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Nikon

NIS-Elements Advanced Research

User's Guide (Ver. 2.34)

Thank you very much for choosing Nikon.
This manual explains installation and use of the NIS-Elements Advanced Research. For trouble-free operation, read this manual before using the program.

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Nikon has carefully prepared this manual. However, we make no expressed or implied warranty of any kind and assume no responsibility for such errors or omissions.
Be sure to read the instruction manuals for the microscope and PC you plan to use with the NIS-Elements Advanced Research.

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To run NIS-Elements Advanced Research optimally, the following specifications are recommended.
Recommended PC Environment:
- CPU: Pentium IV, 3.2 GHz or faster
- RAM: 1GB or higher
- Operating system: Windows XP SP2 (English)
- Hard disk space: 600MB of available hard disk space for installation
- Display settings: 1280 x 1024 (True Color mode)
- User rights: Administrators for installation and operation

*Operation cannot be guaranteed on all computer models. For futher information, contact your nearest Nikon representatives.

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AR Chapters and paragraphs marked by this symbol describe features available only in the Advanced Research version of NIS-Elements.

Installation and Startup

NIS-Elements Installation

Quick Start

Quick Start

- Insert the installation CD in the CD-ROM drive. A splash screen automatically appears.
- Install the selected NIS-Elements software version, additional modules, and device drivers.
- Plug in your HASP key into the USB port of your PC.
- Run NIS-Elements

The CD-ROM Content

The CD-ROM Content

The CD-ROM contains the NIS-Elements software itself, Windows system drivers for all supported cameras, drivers and utilities for HASP, documentation in PDF format, a sample image database, and sample image sequences for additional modules.

Installation

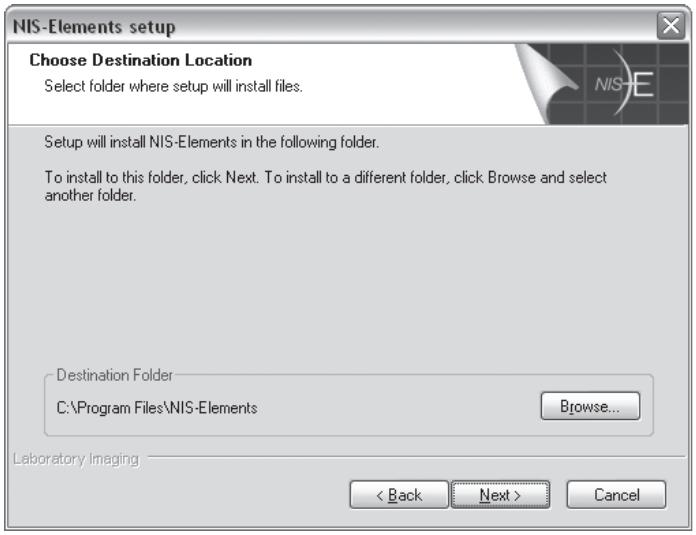
The installation process

Note that you have to possess the administrator rights to your computer to be able to install NIS-Elements successfully. When the installation CD is inserted a splash screen appears.

Specifying the software version

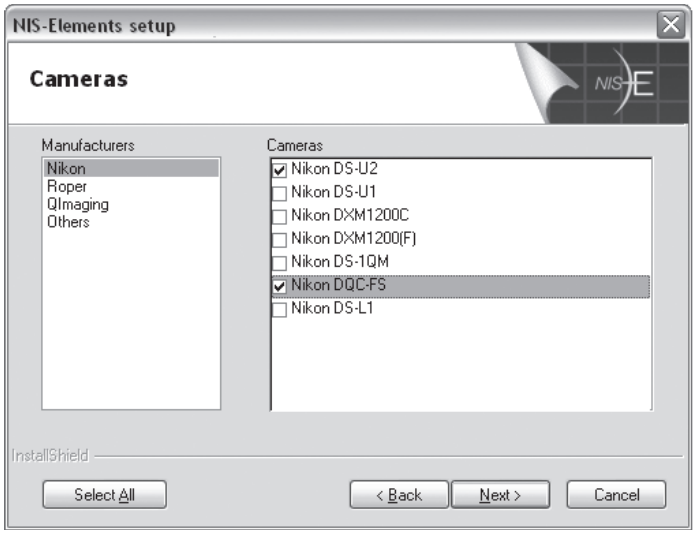
Select the software version to be installed. Select the one you‘ve got the license for and that is properly coded in your HASP hardlock key. The welcome dialog box appears. Click **Next** to continue.

Installation directory


NIS-Elements setup
Choose Destination Location
Select folder where setup will install files.
Setup will install NIS-Elements in the following folder.
To install to this folder, click Next. To install to a different folder, click Browse and select another folder.
Destination Folder
C:\Program Files\NIS-Elements
Browse...
Laboratory Imaging
< Back
Next >
Cancel

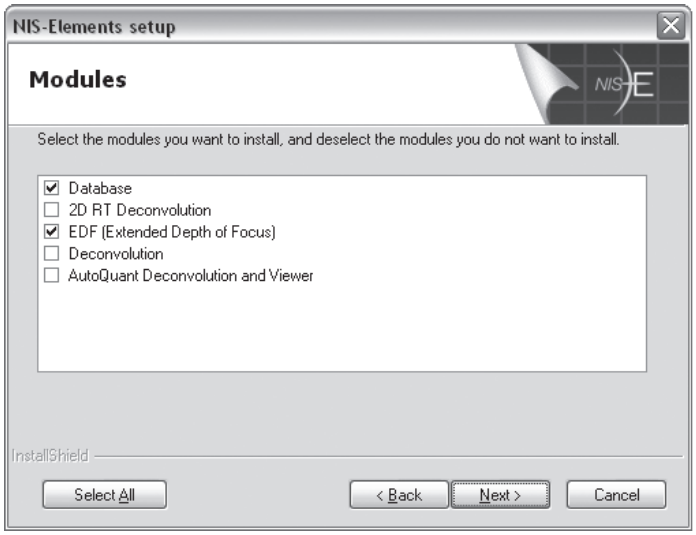
Define the folder where the NIS-Elements software should be installed. We recommend to use the predefined directory. If you want to change the directory, press the **Browse** button and select a new one, otherwise click **Next**.

Selecting cameras



Now select the cameras you want to use with NIS-Elements.

Selecting modules

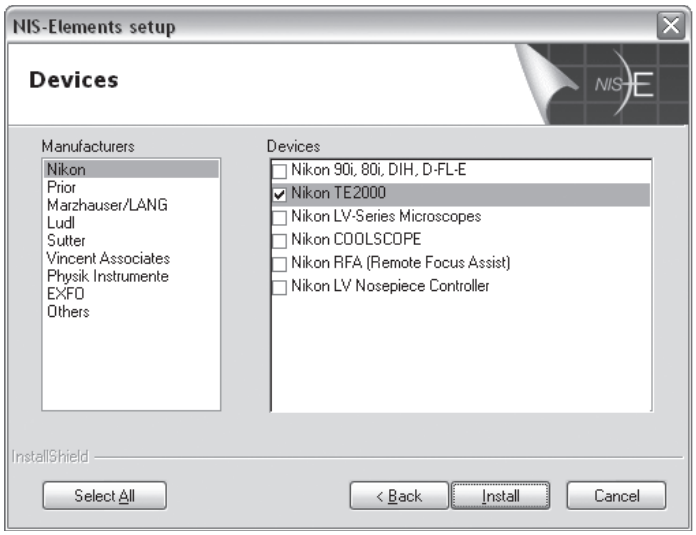


If your licence contains some additional modules besides the NIS-Elements base software, please select them in this window.

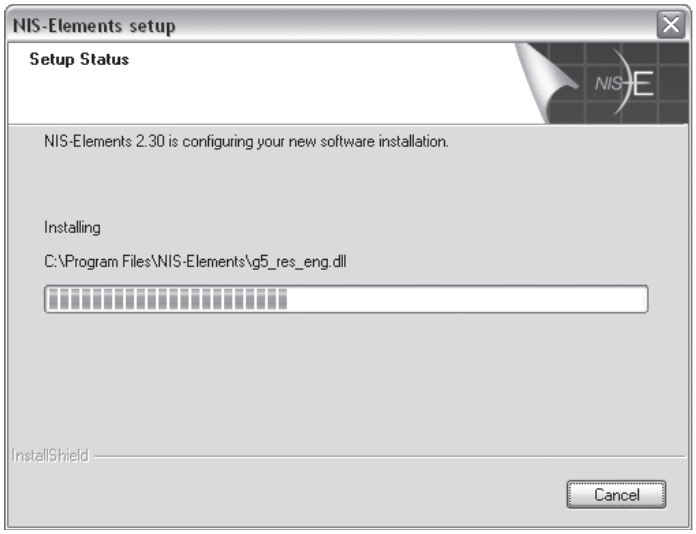
Note:

Any module selected will be installed along with NIS-Elements automatically. However, you might not be licensed to use it. The module will run after you get a corresponding code registered in your HASP key.

Selecting devices

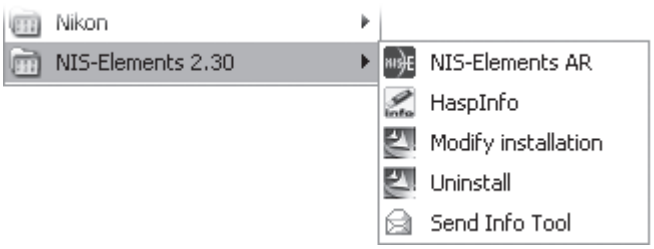


If your licence contains some device controllers listed in this window, please select them. Finish the installation by clicking the **Install** button.



The program group

The Setup creates the NIS-Elements program group with the following items: HASP key information, NIS-Elements shortcut, Modify Installation (for adding hardware drivers, modules, etc.), Uninstall procedure, and the Send Info Tool. A shortcut is created on the desktop. These changes affect all user profiles of your local Windows system.



Note:
Clicking the Uninstall command deletes all installed files from disk, and removes the NIS-Elements program group from Windows Start menu as well as the desktop icon.

Additional installations

Additional Module/Device installation

You may need to install a device or additional module after the NIS-Elements main system installation.

- Go to **Start menu>Programs>NIS-Elements** program group.
- Select the **Modify Installation** command.
- Follow the install shield instructions.Check the checkboxes by the items you would like to add.
- Finish the installation.

Installing sample database

Sample Database installation

If you chose to install the Sample Database, a new subdirectory „Databases“ is created in NIS-Elements installation directory (e.g. C:\Program files\NIS-Elements\Databases\...). The „Sample_Database.mdb“ file is copied in there along with database images (stored in subdirectories). An administrator username/password to access this database is set to:

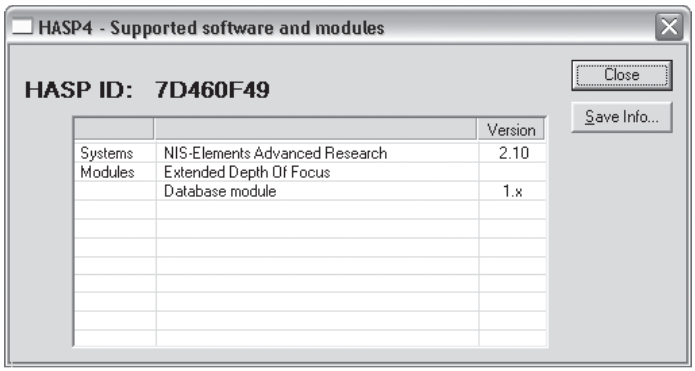
- Username: „sa“
- Password: „sa“

Software copy protection

The software copy protection

The NIS-Elements software is delivered with a HASP hardware key.

The HASP key contains information about the SW licence and allows users to run the corresponding software. Please connect the USB HASP after NIS-Elements installation. The utility called „HASPinfo“ (Help menu) is installed to the NIS-Elements directory. It enables the user to view information about the actual software licence:



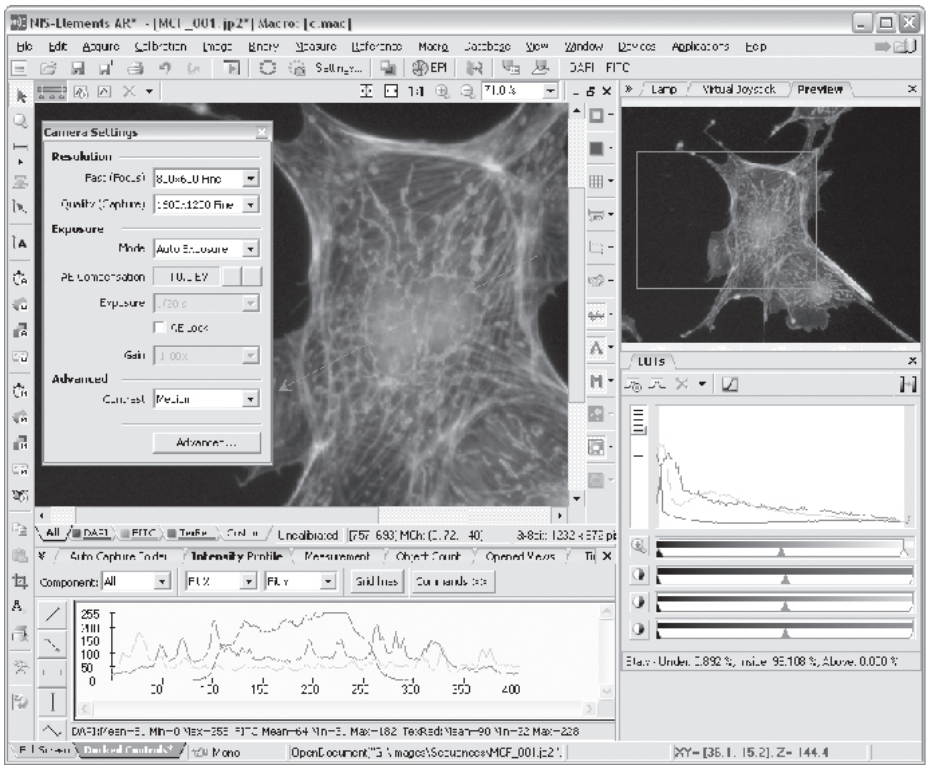
Application Appereance

NIS-Elements Screen

Screen items

The following items appear on the screen:

- **The menu bar** containing pop-up menus with sets of commands.
- **Two main toolbars (horizontal and vertical)**
- **Document toolbars (right and top)** enabling to customize the images view. See its description in the Document structure chapter.
- **Docked control windows** - see more description of docking windows in the Layout Manager chapter.
- **The status bar** displaying useful information.



Main toolbars

Horizontal toolbar

- **Open** file shortcut.
- **Save** file shortcut.
- **Save Next** shortcut.
- **Print Image** shortcut.
- **Undo** button.
- **Redo** button.
- **Show live window** displays a new document window with live signal from camera.
- **RAM buffer ON** - please see the Capturing to ring buffer chapter.
- **Capture to RAM buffer** - please see the Capturing to ring buffer chapter.
- **Background correction ON** is a shortcut to the Acquire>Backgroun Correction ON/OFF command.
- **Explore optical configurations** button shows the optical configurations manager.
- **New optical configuration** runs the optical configuration wizard.

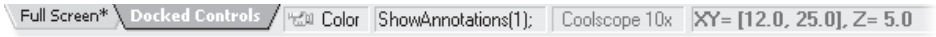
Vertical toolbar

- **Pointing Tool** enables to drag the image.
- **Magnifier Glass** - interactive magnifier tool to examine image details.
- **Length** measurement tool for interactive length measurements.
- **Area** measurement tool for interactive area measurements.
- **Taxonomy** tool for sorting objects in the picture into classes.
- **Counts** tool for counting objects in the picture.
- **Radius** tool for measuring radius of a circle.
- **Semiaxis** tool for measuring semiaxes of an ellipse.
- **Angle** tool for measuring angles.
- **3D Measurement** tool measures length accross Z sequences (req. EDF module).
- **Insert Arrow** tool for inserting annotations.
- **Insert Text** tool for inserting text annotations.
- **Capture Time Lapse** automatically or manually.
- **Capture Z Series** automatically or manually.
- **Capture Multichannel** automatically or manually.
- **Capture Multipoint** automatically or manually.
- **Real Time EDF** performs Z series acquisition and EDF in one step (req. EDF)
- **Copy** copies the current image to memory.
- **Paste As New Image** creates a new document and inserts the image from memory.
- **Crop** tool for cropping images.
- **Run Macro** command runs the current macro.
- **Close All documents** shortcut.
- **General Options**
- **ToolBar Setup**

Status bar

Status bar

The status bar at the bottom shows the following information (from left to right): available views, a type of selected camera, the function corresponding to the last command used, active objective name, and current coordinates of a stage and a Z-drive (if connected).



Layout Manager

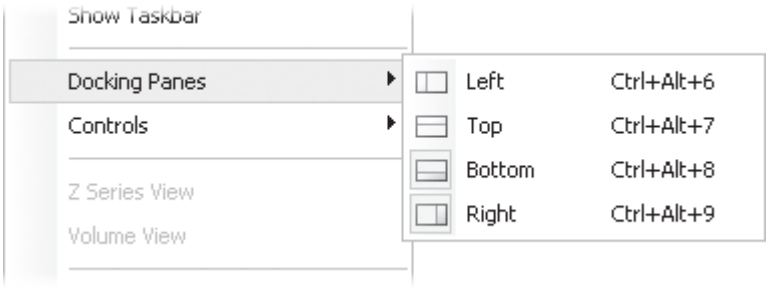
There can be a number of control windows (Camera Settings, Measurement, Histogram, LUTs, etc.) displayed within the application window in several different ways, floating., docked inside a docking pane, in tab or caption style..

Displaying docking pane

To display a docking pane

Docking panes are empty square spaces inside the application window, where you can place („dock“) the control windows. They can help to keep the screen „tidy“. There is one docking pane available on each side (Top, Right, Bottom, Left).

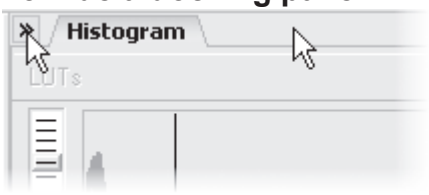
- Go to the View>Docking Panes submenu and select the pane you would like to display.
- The docking pane appears, either empty or with some window(s) docked.
- Repeat this task to display more panes.



The same submenu can be also displayed by right-clicking into the empty application screen.

Hiding docking pane

To hide a docking pane



Either click the arrows in the top left corner, or double click the empty pane (not the docked control window). The pane minimizes to a stripe by the edge of the application. It can be recalled to its original size by double clicking it.

If you would like to close the pane completely, minimize it and press the cross button, or you can right click the pane and unselect the **Docking View** option.

Shrink/Expand docking panes

To shrink/expand docking panes

Having more docking panes opened, a situation where there is not enough room for the control windows can occur. In that case, the Shrink and Expand commands shall be used.

- Right click the pane you would like to shrink/expand.
- A context menu appears.
- Select the Expand/Shrink command.

The commands Shrink/Expand Top, Right, Bottom, Left are available. When one of the panes shrinks, the neighbouring pane expands to the emptied corner and vice-versa.

Control windows

Control windows

The following control windows can be displayed within the application screen. They can be floating, or docked inside the horizontal or vertical docking panes:

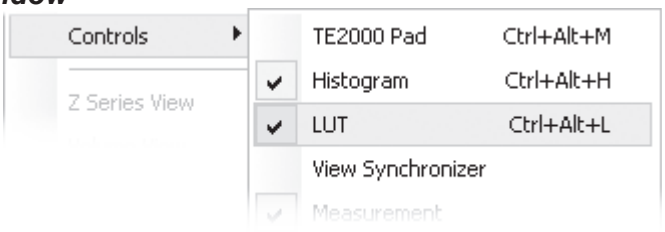
- **3D Measurement* (horizontal, req. EDF)**
- **Auto Capture Folder (horizontal)**
- **Camera Settings* (vertical)**
- **Filters and Shutters* (vertical)**
- **Histogram (vertical)**
- **Intensity Profile (horizontal)**
- **LUTs (vertical)**
- **Lamp* (vertical)**
- **Measurement (horizontal)**
- **Microscope Control Pad* (vertical)**
- **Nosepiece* (vertical)**
- **Object Count (horizontal)**
- **Opened Views (horizontal, vertical)**
- **Preview (vertical)**
- **ROI Statistics (vertical)**
- **RT Deconvolution* (vertical)**
- **Time Measurement* (horizontal)**
- **View Synchronizer (vertical)**
- **Virtual Joystick* (vertical)**
- **Volume Measurement (horizontal, vertical)**
- **Z Profile* (horizontal, vertical)**

** - this control window is optional. A proper software licence or a correct device connected is required in order to display it.*

Displaying control window

To display a control window

- Go to the **View>Controls** submenu and select the desired control window.
- The control window appears floating on the screen.
- To dock (and undock) it, double click its caption.



It is also possible to display a control window docked. Right click inside a docking pane and select the one of your choice. If the window is already opened somewhere (e.g. in the opposite pane or floating), it closes and moves to the new destination.

Closing control window

To close a control window

- Click the „cross“ button on the right side of the active window caption.
- If the window is docked, you can also right click its caption and uncheck the appropriate window.
- The control window closes.

Caption styles

Tab or caption style?

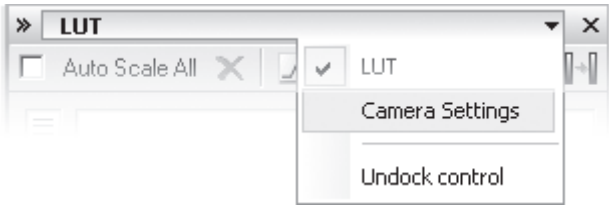
More control windows can be docked in the same place while only the front one is being visible. There are two styles of displaying these windows, Tab and Caption.

Tab Style

- Besides the current control window, the other window tabs are visible.
- Switching the windows is a one click operation.

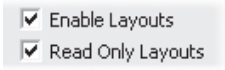
Caption Style

- All docked windows except the current one are hidden.
- The switching and undocking of windows is done via the menu that appears when you click the arrow button.



Managing layouts

To manage layouts via the layout manager



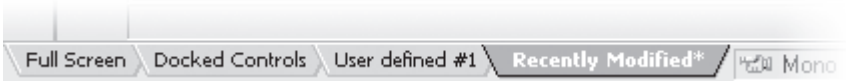
The application can operate in two modes: supporting layouts or not. To switch the layouts mode ON/OFF, reach the General Options tab by double clicking the NIS-Elements caption. In the Appereance section, there is a check box that enables/disables the mode.

When you start the application with the Layouts mode ON, blue tabs representing active layouts appear in the bottom left corner. The Full Screen and Docked Controls layouts are placed by default. Other layouts can be added and managed via the Layout Manager.

If the „Read Only Layouts“ box is checked, no layout changes are saved during work or on exit.

Creating new layout

To create a new layout



- Modify the current layout to suit your concept of work.
- The asterisk appears next to the layout name (to indicate it has been modified).
- Right click the layout tab and select the **Save Current Layout As** command.
- Write the new layout name and confirm it by OK.
- A new tab appears and the layout is saved to the list of layouts.

If you do not need to create a new layout but would like to save the changes made, just right click the current tab (asterisk-signed) and select the **Save** *command from the menu.*

Discarding changes

To reload previous layout settings

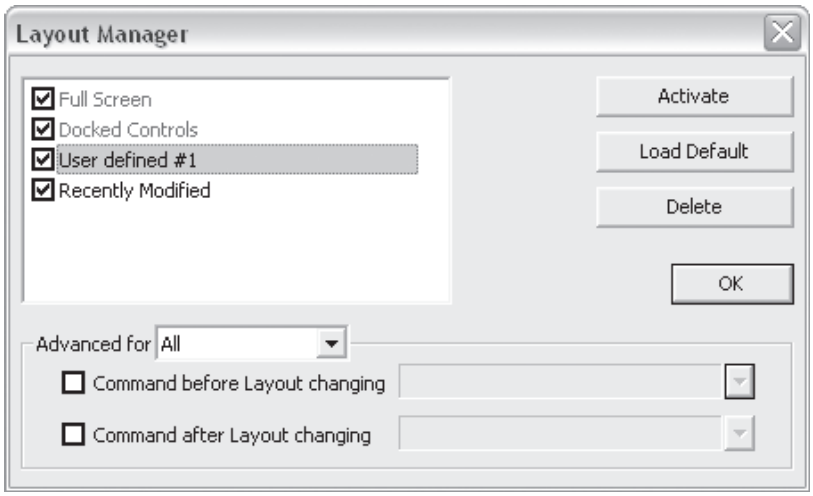
Unwanted changes of a layout may be made. Mostly, it can be fixed by the **Reload** command.

- Right click the asterisk-signed (recently modified but not saved) tab and choose the **Reload** command.
- The application restores the last saved state of the layout.

Using layout manager

Layout manager

Right click any layout tab and select the Layout Manager command. The Layout Manager appears.



- The check boxes on the left indicates the layout visibility (displayed/hidden)
- The **Activate** button makes the selected layout active (current)
- The **Load Default** button has the same functionality as the Reload command described above. Except when applied to the Full Screen and the Docked Controls layouts where it loads the original settings - so they look just the same as after the program installation.
- The **Delete** button deletes the selected layout. The first two layouts cannot be deleted.
- Advanced settings enable to run a command or a macro right before and after a layout change. It can be assigned to one or to all the layouts.
- The **OK button stores all changes and closes the Layout Manager.**

Organizer

Organizer structure

Apart from the main application mode used for capturing and image analysis, NIS-Elements provides a special **Organizer** mode.

Organizer was designed to ease the work with image files and databases. Clicking the **Organizer** button located in the upper right corner of the NIS-Elements application window or pressing **F10** activates Organizer.

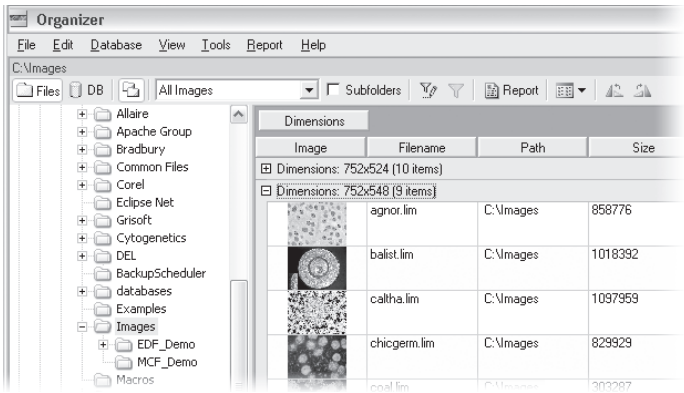
The screen is primarily divided into two identical panes. Each pane can be switched into two modes: the Files mode and the Database mode. To switch from one pane to the other one use „Next Pane“ command from the View menu. To copy files from one pane to the other one simply drag selected images to the other pane.

Files - This button switches the pane to show a directory tree and images from the selected folder (and its subfolders, when selected - see below). It is called the **Files view**.

DB - This button switches the pane to show the database structure and lists images from the currently selected database table. This is called the **Database view**.

Files view

Features available in the Files view



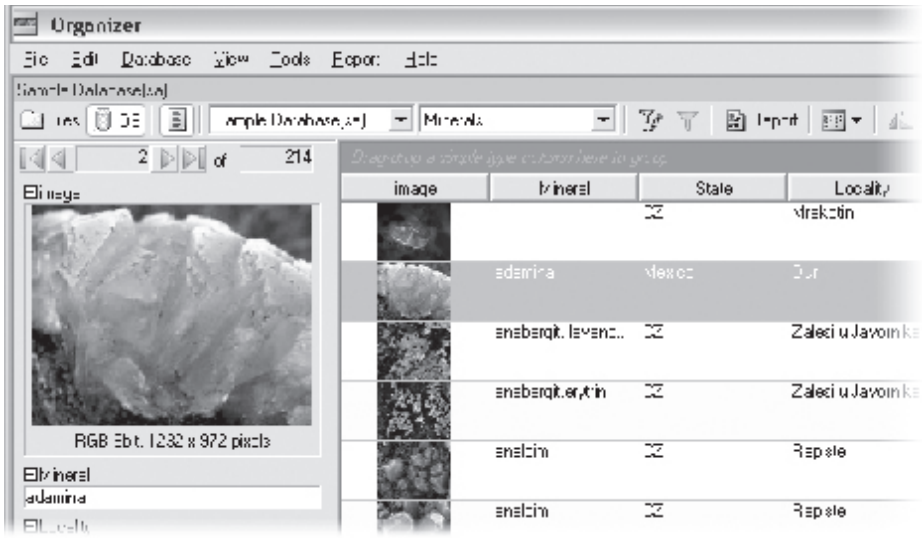
- This button toggles the display of the directory tree. You can switch it off to get additional space to display images.

JPEG2000 - In this combo box it is possible to set the file type to be displayed. You can select only a particular extension or show all files.

Subfolders - If this check-box is selected all images from the included subfolders are shown.

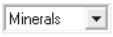
Database view

Features available in the Database view



- This button toggles display of the database navigation and detailed information about the selected image. You can switch it off to get additional space to display images.

- This combo-box displays the active connection point or enables to select another defined connection point.



- This combo-box selects the database table and also indicates the current table.

Filtering images

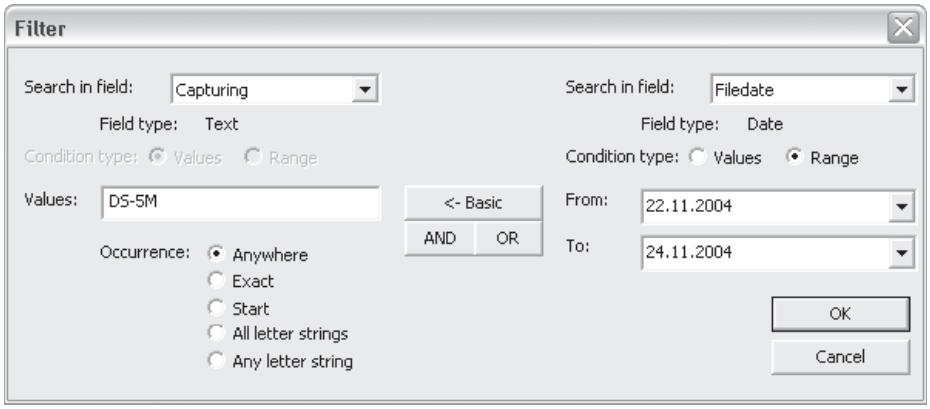
Image filter

Both views (File and Database) have an option to use **filters**. It enables you to display only such images, that match the criteria given.

- This button activates the filter.

- Pressing this button invokes the filter setup dialog box.

The filter can be used in two modes:



Basic filtering

Basic mode

This mode enables you to view files that match one defined condition.

Advanced filtering

Advanced mode

This mode enables you to define two conditions with a relationship defined between them. Either select **OR** to display files with the properties matching at least one given condition, or select **AND** to display files the properties of which match both conditions at once.

First, select the field, where NIS-Elements should search for a given expression. When using a filter in the Files view, there are fields from file properties listed. In the Database view, the listbox shows the names of fields from the currently selected database table.

If a selected field is of a numerical type (e.g. Size, Calibration, File date etc.) you can specify, whether you want to find the exact value or a value in a given range (both displayed in the picture above). This is selected by the **Condition type** radio button.

- **Anywhere** - If a given sequence of characters is found anywhere in the sequence of characters in the field, the system will evaluate it as a match. E.g. you have entered **set** to the values edit box. The filter will select records with field values: **set**, re**set**, **set**tings, pre**set**ed...
- **Exact** - If the given sequence of characters exactly equals to the content of the field, it is evaluated as a match. E.g. if **set** is entered, fields containing the **set** value will only match.
- **Start** - If the entered string is found at the beginning of a field, it is displayed by filter. E.g if **set** is entered, fields containing **set**, **set**ing **set**up are selected.
- **All letter strings** - It is possible to search for more expressions. These should be entered separated by commas. If you want to enter an expression with a space, insert it into

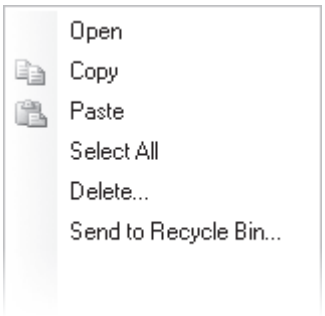
quotes. If this option is selected, only records in whose fields all of those expressions (anywhere) appear are selected.

- **Any letter string** - This option is for entering multiple expressions as above, but this time every field with an occurrence of at least one from the given expressions is matched.

Operations with images

Operations with images

To open an image from the **Files** view, double click it. NIS-Elements will display it in the main mode. To select multiple images, either click on the first and the last image holding the **Shift** key (continuous group selection) or click individual image names with the **Ctrl** key down to select more files that do not appear together.



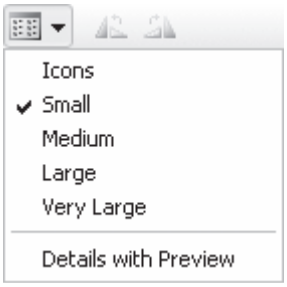
You can copy one or more selected images from one folder to another only by dragging and dropping. This can be used to ease inserting of images to the database too. Simply drag the image from a folder and drop it onto the pane, where the database table is opened. To delete selected images press the **Delete** key.

All those operations and some other can be invoked also from the context menu, which appears each time you right click on the image thumbnail:

Displaying thumbnails

Thumbnail displaying options

You can adjust the way images are shown in the panes. The menu can be invoked in the main bar, where you can select the size of displayed image thumbnails. Selecting the **Details with preview** will display only one image per row with additional information about the image (image properties, if the images are in folder or database fields, when viewing database records.)



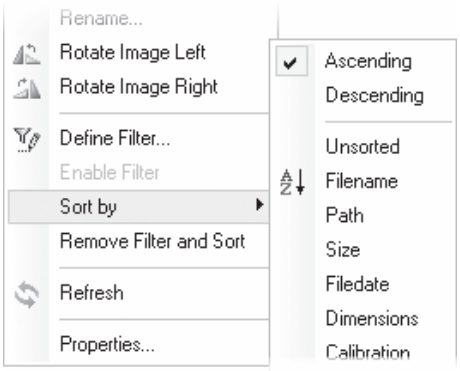
There is a possibility to rotate images which have wrong orientation. It doesn't affect just the display, but changes the underlying image data too.

- Rotates image (or more selected images) left.

- Rotates image (or more selected images) right.

Images sorting

Sorting of images



To adjust the order of displayed images, right click anywhere in the pane. A context menu will appear. Move to the **Sort by** command - the submenu with several possible selectable criteria will appear. If the sorting is already active, the icon is displayed on the left side of the selected sorting criteria (images are sorted by their filenames).

Images grouping

Grouping of images

To better arrange the view of images one can use the grouping of images. Drag the column name bar to the grouping bar (right above the column name bars). All files with matching field values of that column will be grouped together. This can be undone by dragging the column caption back. See the example **Files View picture** above (the Dimensions column is grouped).

Adjusting organizer layout

Adjusting the look of Organizer

The pane size is adjustable. To resize it move the mouse cursor to the dividing in the middle. The cursor becomes an arrow with two tips. Press the mouse button and move it to the new position.

- Resizes the panes to achieve the same size of both of them.

- Resizes the pane to its maximal/minimal size (one pane is then displayed on the whole screen).

Setting the Hardware Up

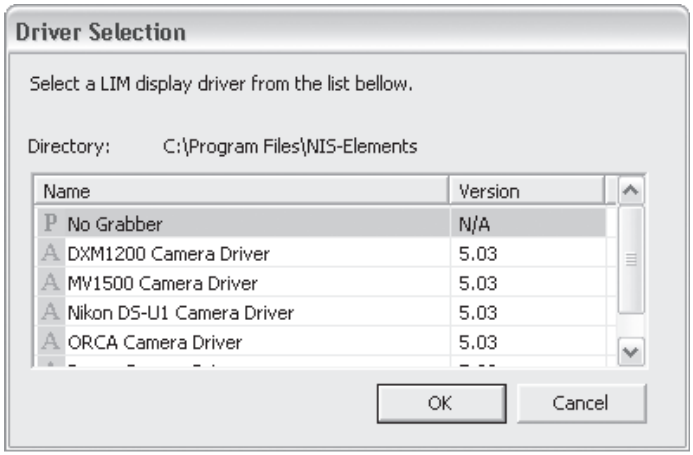
Cameras

This chapter is dedicated particularly to users running the active version of NIS-Elements - equiped with a camera. Let‘s assume the camera works properly, is connected to the system with proper system drivers installed and running (if required by camera).

Selecting driver

Selecting the driver

You will be asked to select the camera driver everytime you launch NIS-Elements. You can change the driver later, using the **Select Driver** command from the Acquire menu. Choose the driver that matches your camera:



Selecting camera

Selecting the camera

Color cameras can be used in a monochromatic mode. The actual camera type (color/mono) can be selected in the Acquire menu by choosing the **Select Camera** command. Confirm the choice with **OK**.

Camera setup

Setting up the camera

Exposure time, camera resolution, and other camera-specific features are adjustable from the **Camera Settings** window. To invoke it, press the Camera Settings button located on the left tool bar, or use the identical command from the **Acquire** menu. A dialog window appears.

Live camera signal

Live signal from camera

This button opens a new document window with the Live-Fast image from camera. New buttons for controlling the camera appear in the document window to control the camera.

Live camera formats

Live formats

A format is a set of attributes of a video signal e.g.: resolution, bit depth, frame rate etc. Live signal is a stream of image data, coming to your computer from the camera in real time. NIS-Elements provides two different modes: **Fast** and **Quality**, each serving a different purpose. The format of both modes can be set in the Camera Settings dialog box.

This tool bar button runs the **Fast** live mode. It is optimized to give as many frames per second as possible in low resolution. This mode increases gain and uses short exposure times.

This tool bar button runs the **Quality** live mode. It produces pictures in high resolution, but the frame-rate is low. This mode sets the gain as low as possible and extends the exposure time. This mode is applied every time the **Capture** button is pressed:

Capturing images

Capturing Images

Although it is possible to perform some procedures directly on the live image, other image operations require **captured** or **frozen** images. If you try to perform such operation when the live image is active, NIS-Elements automatically freezes the image. Let‘s see the difference between **Capture** and **Freeze** functionality:

This is a **Capture** button. When pressed, the camera exposure runs till the end, and the next frame is captured and displayed on the screen (the first frame with the complete exposure after you have pressed the **Capture** button).

The **Freeze** button interrupts the camera exposure, and displays the very last complete frame.

If you are using the fast mode and press **Capture**, NIS-Elements will automatically switch to the quality mode to capture the image. When the image is captured, it is opened on the screen as a new document.

Configuring Attached Hardware

Introduction

Typically, laboratory computer image analysis systems consist of a computer, a camera, and a microscope equipped with all necessary accessories (objectives, filters, shutters, light, various changers, etc). Most of the mentioned microscopic hardware is often motorized and therefore can be controlled by NIS-Elements. Thanks to the NIS-Elements concept, it is possible to integrate single settings of all these devices into one compact set called an **Optical Configuration**.

New optical configuration

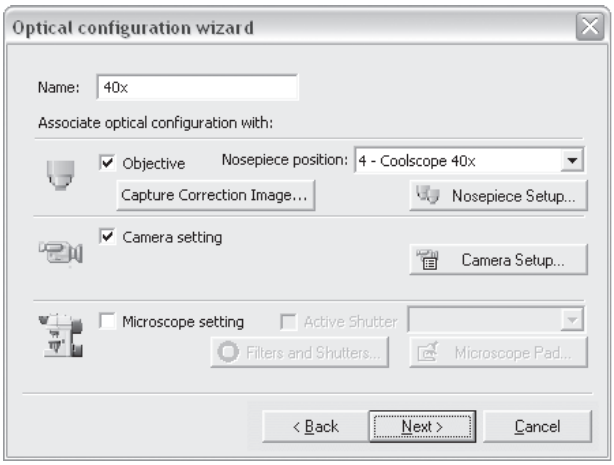
Creating New Optical Configuration

Please check that all your devices (microscopes, cameras, etc.) that you want to associate the new optical configuration with are properly attached to the system and working. Insert a calibration slide with reference objects of known sizes.

Optical configurations wizard

Start the Optical Configuration Wizard

Choose the **New Optical Configuration** command from the Calibration menu. The Optical Configuration Wizard starts. If a camera is connected, it is automatically set to Live mode.

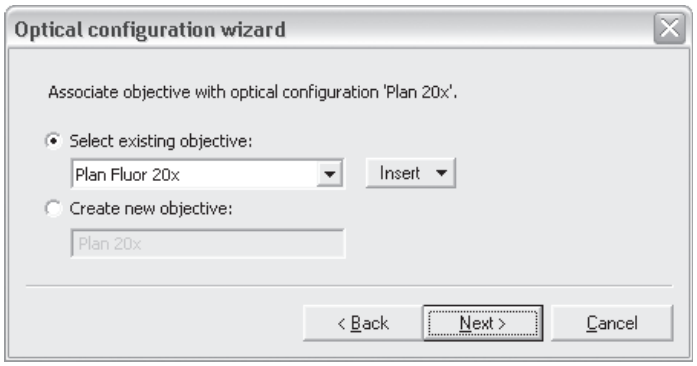


- First type the name of the optical configuration. We recommend to use short descriptive names, e.g. name of the used objective.
- Then select the association to objective, camera or microscope. In case your typical slides differ in brightness from the calibration slide, insert a specimen instead of the calibration slide for this procedure.
- Use the **Camera Setup** button to adjust the camera image input. You can start the illumination correction process by pressing the **Capture Correction Image** button.
- Adjust the Microscope setting by pressing the Microscope Pad button.

Press the Next button (when the Objective was selected) or Finish to continue...

Selecting objective

Selecting Objective



Now, please select one of the following alternatives:

- Select one of already defined objectives from combo box or pick it from the pop-up menu which appears after pressing the Insert button.
- Create a new objective by filling its name in.

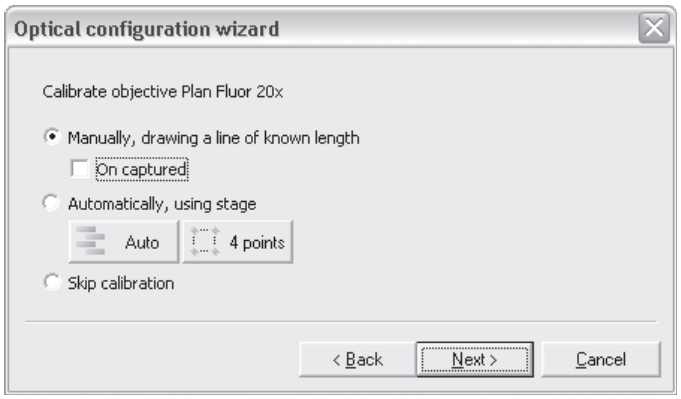
Press the Next button to continue...

Objective calibration

Objective Calibration

All digitized images consist of a number of small rectangular elements called pixels. A calibration assigns real size to one pixel, so correct measurements can be performed. Each calibration depends on the associated magnification, therefore it is necessary to create a calibration for each objective. It is possible to assign names to objective calibrations and to store them.

Select one of the following alternatives:



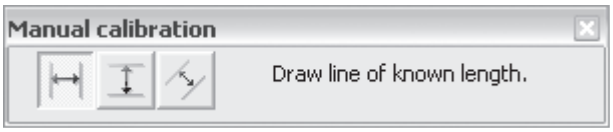
- The **Manual** calibration lets you draw a line into a picture and define its real length (see below). The „On Captured“ check box allows to decide whether a live or captured image is used for a calibration.
- Having a motorized microscope, there are the **Auto** and **4 points** alternatives available.
- Skip the calibration, if the selected objective (selected in the previous step) has been already calibrated.

Press the Next button to continue...

Calibration - manual

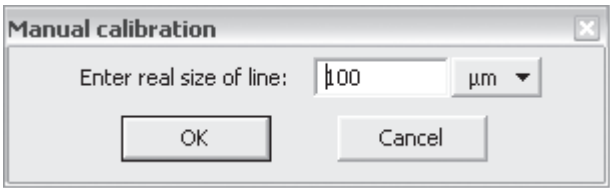
Manual Calibration

This step is dedicated to the manual calibration.



- The distance is defined by placing lines (Horizontal, Vertical, Parallel) in the image. First, choose the lines orientation, then click into the image to place the first line. Place the second line in the intended location by another click. You can modify the line position while holding the mouse button, not later.
- When Parallel lines are selected, first define the beginning of the first line. The next click defines its orientation and length. When satisfied, end the first line creation by right-click. The second line can be placed by another click into the image, this time to adjust the distance from the first line only. The process is completed again by right-click.

The following dialog box appears...



- Now enter the real distance between the two drawn lines and select correct units.

Press the OK button to continue...

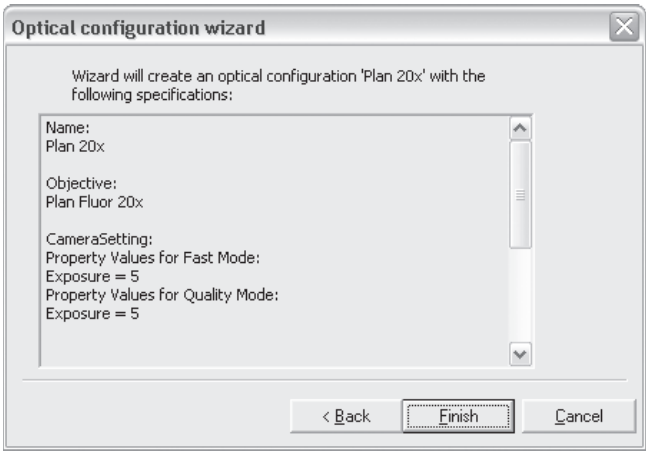
Calibration - automatic

Automatic Calibration (the Auto method)
This method is fully automatic. NIS-Elements moves the motorized stage, acquires two images, and calculates the calibration from the shift of the images.

Automatic Calibration (4 points method)
The system draws four points on the screen (subsequently) and asks user to move some significant part of the specimen by joystick to match the points. After all four steps are completed, the calibration is calculated from the moves of the stage.

Completion

Optical Configuration Completion



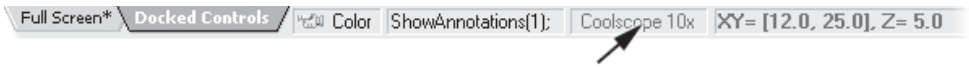
The last status window informs you about the parameters of the optical configuration. Click Finish to complete the process.

- You can create more calibrations by repeating the above procedures.
- The calibrations are stored to registry immediately and can be exported to a xml file (Calibration > Optical Configurations > Backup).
- The selected calibration is maintained. It is applied during any image input from the camera.
- The selected calibration remains unchanged even if a stored image together with its (different) calibration would be loaded. After the camera input has been renewed the formerly chosen calibration is restored.
- It is possible to adjust the created calibration. Select the **Calibration menu > Optical Configurations** command.

Selecting optical configuration

Selecting Optical Configuration

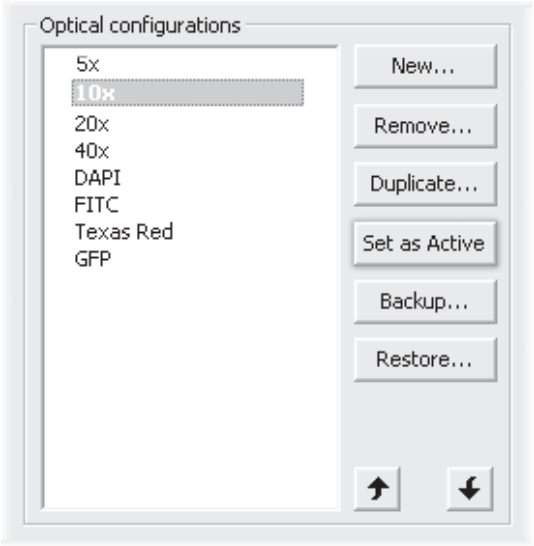
The active objective is displayed on the Status bar:



Buttons representing optical configurations are placed on the main toolbar. This feature is set as default but it is possible to decide independently for every single Optical configuration whether to show its button or not. Pressing these buttons is the shortest way to switch among the configurations.

The other way is to invoke the Optical Configurations window (This can be done by selecting the **Calibration Menu > Optical configurations** or by right-clicking the active objective name on the status bar, and selecting this command from pop-up menu). The Optical Configuration dialog window appears...

Simply select the configuration you want to use, and click the Set as Active button.



Recalibrating captured images

Recalibrating captured images

All images captured while a calibrated optical configuration is selected are calibrated automatically. If you need to change their calibration or you want to calibrate the image acquired via scanner or transferred from digital camera, use the **Recalibrate Document** command from the Calibration menu.

Recalibration is done the same way as the Manual Calibration described above.

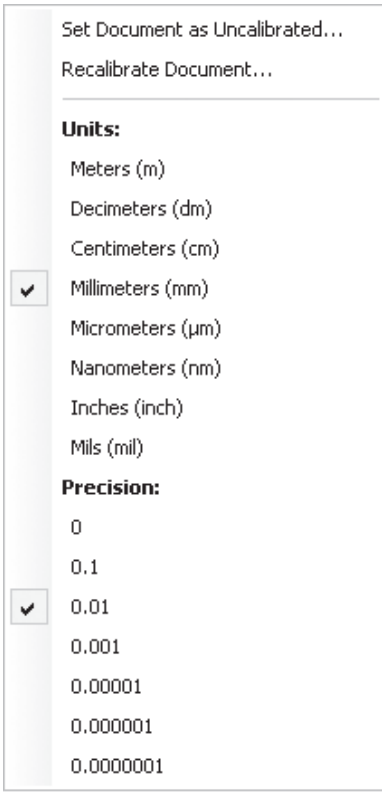
Setting active units

Setting Active Units

NIS-Elements supports following units: pixels, nanometers, micrometers, millimeters, centimeters, decimeters, meters, inches, and mils. If the image is uncalibrated, pixels are the only units available. In case of a calibrated image, it is possible to select the desired units. All values (e.g. measured length/area) are then displayed in the corresponding units. There are two ways how to select the desired units:

1) Right click on the document **status bar** section with the information on image dimension and color bit depth. A pop-up menu will appear. Select the units.

2) Or, click the **Current unit** button located in the **Optical configurations** dialog window and select the units.



Capturing Images

Images Capturing

Having the whole hardware system set up and NIS-Elements installed, you can start capturing images. There are several ways of acquiring images, some of them specialized needing specific hardware. The easiest way of all is to capture a single image:

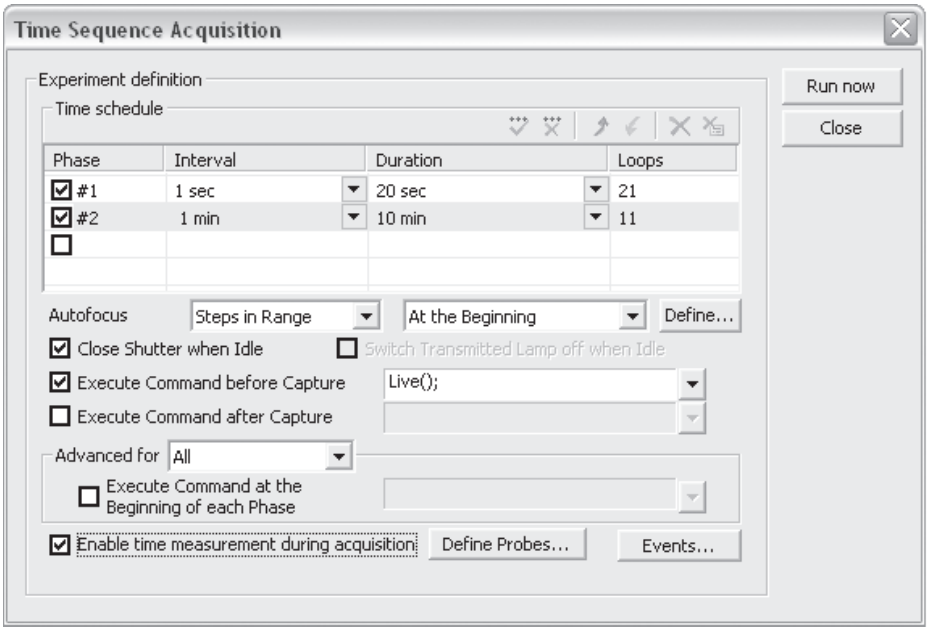
Capturing single images

Single Image Capture

- Turn the connected camera and other devices ON and start NIS-Elements. Select the corresponding camera driver.
- When the application is started display the **Camera Settings** control window - View>Controls>Camera Settings.
- Switch camera to live mode - Acquire>Live Fast - and adjust its parameters to get a clear live image.
- Focus the objective on the scene you would like to capture an image of.
- Switch to the Live - Quality mode and adjust its parameters especially the Exposure time and Format (this mode will be used every time an image is captured).
- Switch back to the Live - Fast mode. It is usual mode for smooth work because it uses less hardware resources.
- **Capture the image** by pressing the capture button on the horizontal toolbar, by invoking the Acquire>Capture command, or using the **Ctrl+-** shortcut.
- A new image is opened and named „Captured“ automatically.

Time Lapse Acquisition

Detailed studying of long-lasting processes is enabled by the time lapse acquisition mode of NIS-Elements. The possible experiment duration is almost limitless. Invoke the **Acquire>Capture Time Lapse** command to set the experiment up:



Timelapse options

Time Schedule

The Time schedule table enables you to define consecutive time phases where duration, interval between single images, and number of images of the phase can be adjusted for each phase. The Interval, Duration, and Loops settings are bound together, so you just need to set two of these parameters. The remaining parameter is calculated automatically.

Auto Focus

Automatic focusing can be used during experiment. You can select from the various **Autofocus** methods that best meets your needs. The nearby combo box determines whether the focusing shall be performed only at the begining of the experiment, at the begining of each time-phase, or before each frame captured. The Define button shows a dialog window where you can define parameters of the selected focusing method.

Special Options

The shutter can be closed between the acquisitions. Just check the **Close Shutter...** box.

The **Advanced for** combo box enables to run command (or macros) at the begining of each loop. You can define a common command (or macro) for all loops of the dimension or set a command (or macro) individually for each loop.

You have an option to run a command* (or a macro) before and after Z-Series. It is possible to enter the command directly, or press the rightmost button to show the context menu, where you can select a command from list (**Command List**), or search for macro files on disk (**Run Macro**).

* - *The Live(); command can be used often. However, in some exceptional circumstances the Live(); command may not work properly. In such case, please, use the LiveNoMsgLoop(); command instead.*

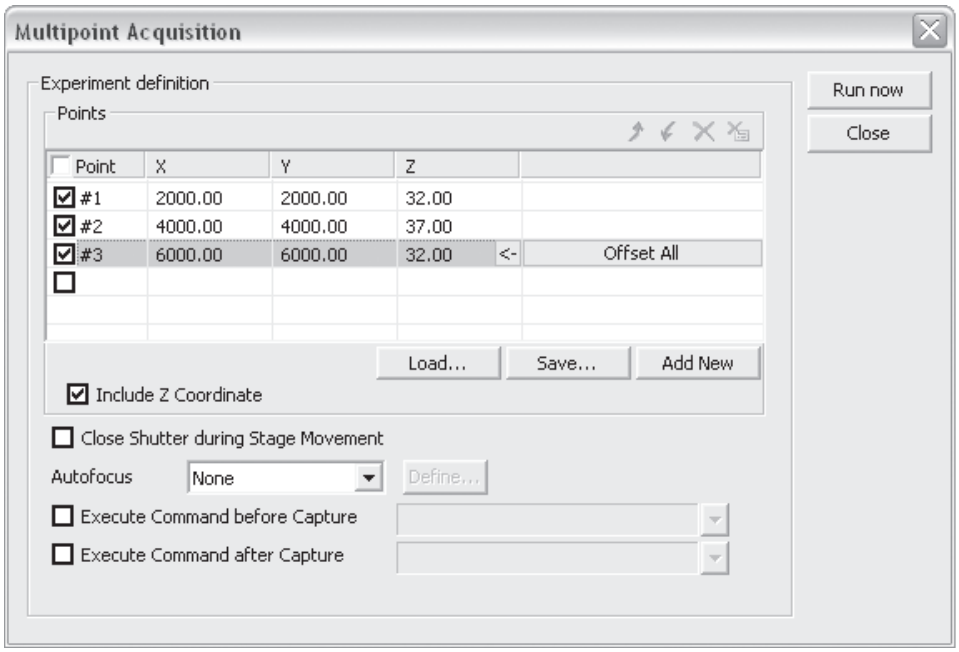
Time Measurement

Time Measurement

You can even **Enable time measurement during acquisition**. When no probes are defined, use the Define Probes button. Please, see the Time Measurement chapter for further details.

Multipoint acquisition

This feature is available when a mototized XY(Z) stage is present in the system and connected properly. An arbitrary array of XY(Z) positions can be defined to be scanned during the multipoint capture experiment. The Z position can be included optionally. The defined array can be saved (and loaded later) to an XML file by the Save button. Invoke the **Acquire>Capture Multipoint** command.



Multipoint options

To define a multipoint array:

- Move the stage to the first point (via a joystick or the „Move“ command).
- Press the **Add New** button. The new line containing current coordinates appears in the list.
- Move to the next position and repeat the steps until you have all the intended points defined.

To change a single Z coordinate:

- Click in the line you would like to change.
- Move the Z drive to the new position.
- Click the „<-“ button.

The XY coordinates of one point cannot be adjusted (unless you delete one and add a new point).

Offset All

This button appears next to the currently selected point. It can shift the XY coordinates of all points in the same way:

- Select one point of the list. (The stage moves to its coordinates automatically)
- Move the XY(Z) stage to a new position (define the offset).
- Press the **Offset All** button.
- The coordinates of all points are rewrited (The same shift as you made is added/subtracted).

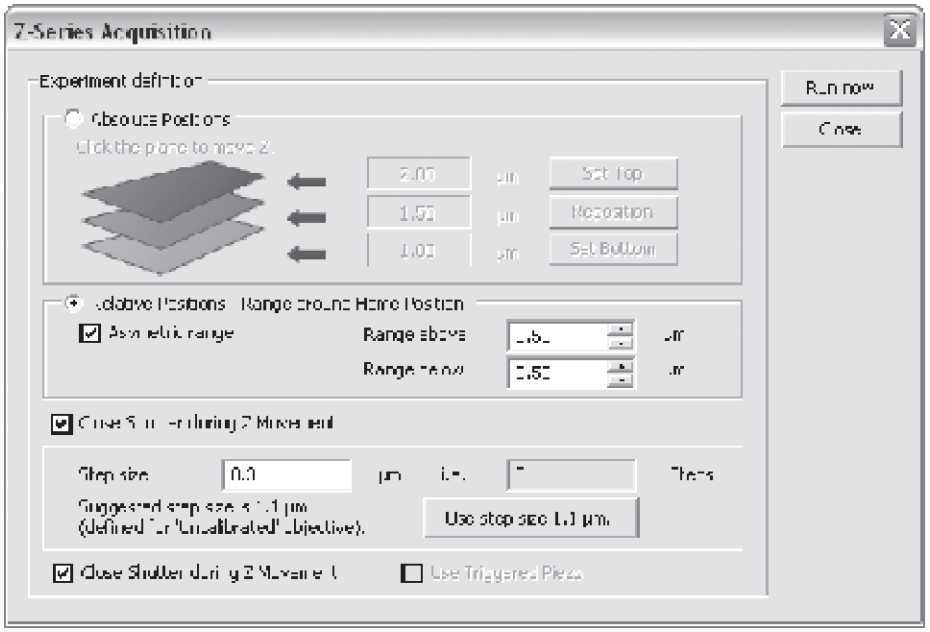
Special Options

Special Options

Please find the description of all the other options in the **Time Lapse Acquisition** *chapter.*

Z Series Acquisition

Capturing images from different focal planes of the specimen can be performed using the **Acquire>Capture Z Series>Capture Automatically** command:



This dialog serves for setting the method of capturing in Z Series. Select between *Absolute positioning* and *Relative positioning*

Z Series options

Absolute positioning

The two methods vary in the way the range is defined. The **Absolute positions** method requires setting of two positions, by moving the stage and pressing the appropriate button. The third position is counted automatically. For example if you define Top and Bottom position, the middle one is set in the midle of these positions. Then if you move the stage and press the Reposition button, all settings are recalculated so the middle value equals the current stage position.

Relative positioning

Relative positions method can be used in **Symetric** (default) or **Asymetric** variation. Symetric captures slices in range, that is same in both (up and down) direction from home stage position. The value in Range editbox represent the whole range of capturing. When selecting the Asymetric range checkbox, two editboxes, for defining individual range in each

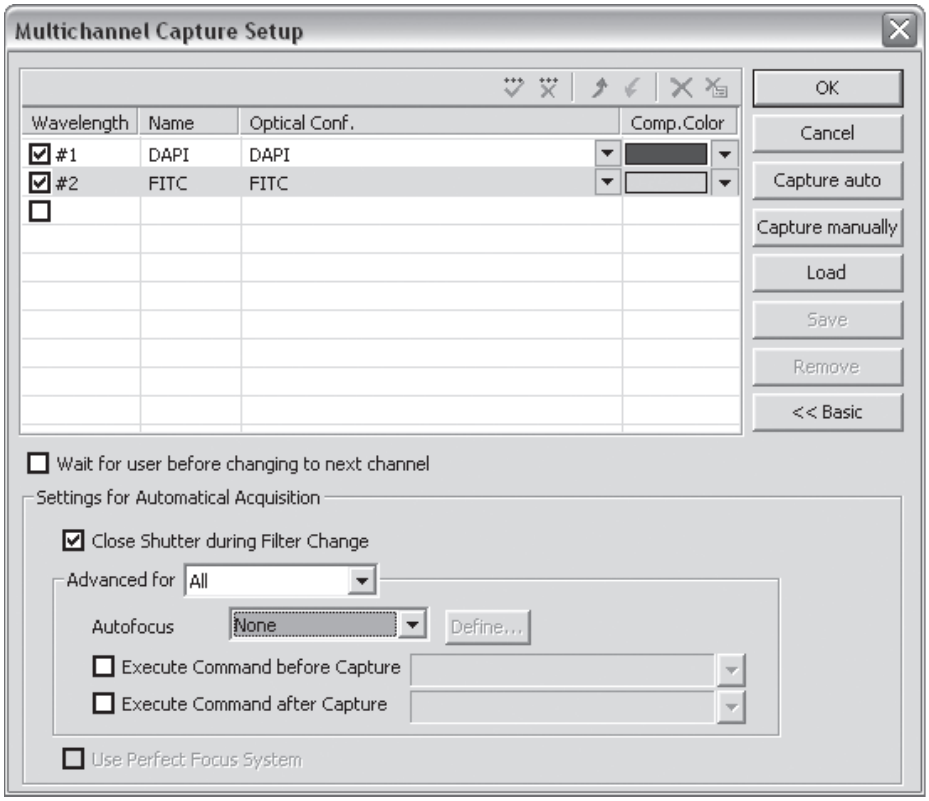
direction. The predefined values are halfs of the range defined in Symetric method.

Step Size

You can define the step between images in Z axis manually, by entering the number of slices or exact step size. It is possible to **Use the** automatically generated **suggestion**, that is computed according to selected objective.

Fluorescence acquisition

Fluorescence (multichannel) pictures can be acquired using the **Acquire>Capture Multichannel Image>Capture Manually/Automatically** commands. However, The Multichannel setup should be adjusted first (the Multichannel Setup command is located in the same submenu):



Multichannel options

Channels Setup

This table defines number and type of captured channels. First set a descriptive name, then select the optical configuration (from combo box) that is to be used for capturing the channel. If any suitable configuration does not exist, you can create a new one, by selecting the **define new** option (when clicking on the down arrow button next to the Optical Configuration name). Comp. color specifies the color tone, in which this channel will be displayed.

Manual Filter Change

The **Wait for user before changing to next channel** option enables you to switch the wavelength filters manually during the multichannel experiment. Use this option if there is no automatic filter changer available.

Autofocus

The **Autofocus** *settings are applied within the automatic multichannel acquisition only. When capturing multichannel images manually, the autofocus is not available.*

Automatic Capture

Automatic Capture

The Capture Automatically command runs the automatic multichannel acquisition procedure according to the Multichannel Setup settings. When the procedure is finished, the resulting multichannel document is opened.

The automatic capture can be performed even you do not have a motorized filter changer. The **Wait for user before changing to next channel** *option must be checked. Then a confirmation dialog box before each channel acquisition appears.*

Manual Capture

Manual Capture

The Capture Manually command opens a new document window with the live image and the Capture button. After pressing the Capture button one channel is captured. The number of channels depends on the Multichannel Setup.

A single channel may be recaptured by the Live button during before the experiment is finished. Select the channel and press the Live button that appears instead of the Capture (at already captured channels).

After all channels are acquired, the Recapture button replaces the Capture button. It enables you to capture the multichannel document again. All previously obtained multichannel data will be lost. When a single channel is selected, it can be recaptured separately.

Capturing to Ring Buffer

Capturing to RAM

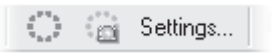
The Capturing-to-RAM technique enables user to record sequences displaying very quick actions, lasting tens of miliseconds. The quality and number of frames in a sequence depends on hardware capabilities of the computer system.

Ring buffer size

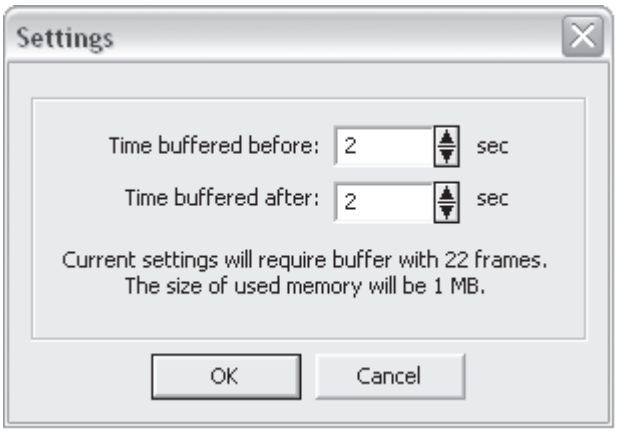
Set the ring buffer size

The technique uses a ring buffer to store temporary data. The ring buffer is a part of virtual memory that is being constantly (and repeatedly) filled with the live image data. Depending on how fast the action to be captured is, you should set the ring buffer size in miliseconds.

- Click on the **Settings** command next to the RAM capture buttons.



- Set the **Time buffered before/after** values. These represent the time before and after you click the RAM Capture button. The whole time interval will be included in the sequence.
- Confirm it by OK.



Buffer ON/OFF

Turn the buffer ON

- Click on the leftmost button of the RAM capture tool bar to enable recording of Live image to RAM.
- The ring buffer functionality is activated.

When the Camera is switched to Live mode, the left-side ring icon begins to indicate the buffer activity. If the RAM Capture button is pressed then, the captured sequence contains frames from the whole time interval before and after the button press. If you press the button while in the Frozen mode or without the time buffer ON, only the **Time buffered after** *sequence is grabbed.*

Capturing a sequence

Capture a sequence

- Press the **RAM Capture** button.
- A new document window containing the captured seqence opens.

Documents, Archiving

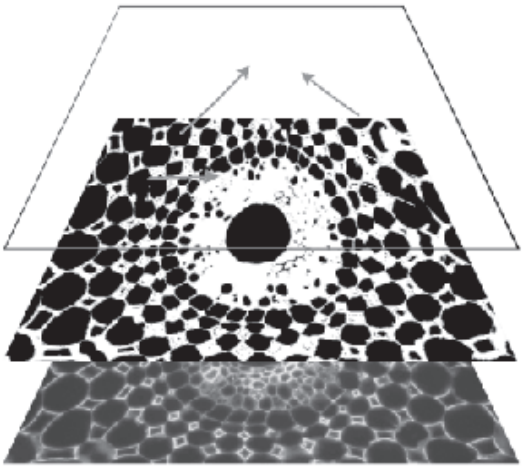
NIS-Elements Document Structure

Image Layers

Image Layers

Each captured image can consist of the following image layers, serving different purposes:

Annotation layer - In this layer, graphical objects and texts can be stored in a vector format. The results of interactive and automatic object measurements, arbitrary text notes and other annotations are stored there.



Binary layer - Pixels in this layer can be found in two states only (i.e. Black/White). A binary image is usually result of thresholding. It is used mainly for performing automatic measurements of the thresholded objects.

Color layer - Contains image data. When you open an image from disk, it is loaded to this layer. It can handle images with the depth of up to 16 bits per color component. The dimensions of this layer determine the view of the other layers.

When saving an image, only the JPEG2000 and ND2 file formats can handle all image layers and are capable of saving them. The other image formats will save the content of the color layer only.

Document types

Document types in NIS-Elements

RGB documents

Images acquired by a color camera typically consist of three components that represent red, green and blue channel intensities. Pixel values for each component range from 0 to 65535 (in 16 bit depth). You can display a single color channel using the tabs located in the bottom-left corner of a document window.

Multi-channel documents

These documents arise usually from fluorescence microscopy. Instead of 3 color components (R, G, B), multichannel images can be composed from many user-defineable color planes. When a multichannel image is opened, the channels panes (bottom-left corner) are not the standard ones (marked with Red, Green, Blue), but are named differetly according to the different colors. The maximum number of channels are 32.

If there is a document that contains more than 8 components, the tabs are replaced by the Lambda dimension, similarly as other dimensions of **ND2 documents** *are displayed.*

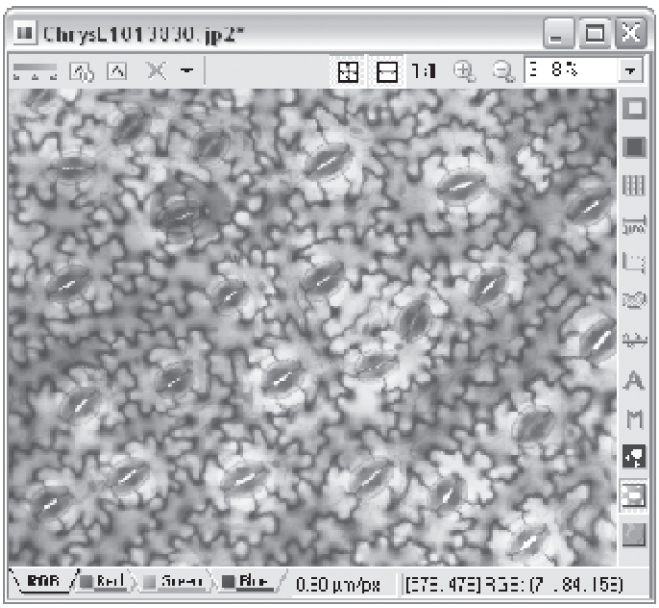
ND2 (N dimensions) documents

The ND2 document is in fact a set of images. There are four types of ND documents depending on the method of acquisition: **T** for timelapse acquisitions, **XY** for multipoint acquisition, **Z** for Z-series (slices) acquisition and **Lambda** for acquisition of a defined wavelength (fluorescence imaging). All these methods can be combined together. In the most advanced case, you can have image data from defined time periods captured in different positions of an XY stage, each image can be displayed in defined numbers of its Z slices and each slice can consist of number of planes, where each plane can be acquired using different light wavelength. All the images captured by these methods can be stored to a single file.

Document window

Document window

Since NIS-Elements supports multiple windows, all controls affecting the view of the image, its layers, and components are located in every image window. Therefore it is possible to control each opened image individually.



Document control buttons

LUTs

- **Enable LUTs** button applies **LUTs** to the image.
- **Keep Auto Scale LUTs** button applies the AutoScale command to the image continuously.
- **Auto Scale** button performs automatic setting of **LUTs**.
- **Reset LUTs** button discards the LUTs settings.

Zoom controls

- **Fit to screen** toolbar button. Adjusts zoom to view the whole image in its maximum magnification. It corresponds to the **Fit to screen command**.
- **Best Fit** toolbar button. It adjusts zoom to view the image as large as possible in NIS-Elements image window. Corresponds to the **Best Fit command**.
- **1:1 Zoom** toolbar button. It adjust the zoom to view the image in its original magnification. It corresponds to the **1:1 Zoom command**.
- Increases magnification. It corresponds to the **Increase command**.
- Decreases magnification. It corresponds to the **Decrease command**.

General controls

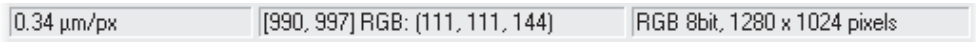
- **Show Probe** button activates the probe. The probe affects histograms, auto exposure and auto white functions.
- **Show Background Probe** toolbar button activates the background probe. Some commands (Subtract Background) uses the BG probe data as reference.
- **Show Grid** button displays the grid for rough measurements.
- **Show Scale** button displays the scale.
- **Show Frame** button displays and applies the measurement frame.
- **Show Measurement ROI** button displays the Measurement ROI.
- **Show Profile** toolbar button displays the intensity Profile window. This tool alows you to specify direction in the image (using arrow) and the graph displays intensity of pixels, that this arrow passes trough.

Right click the icons to invoke a context menu where properties of each tool can be modified.

Document status bar

Status bar

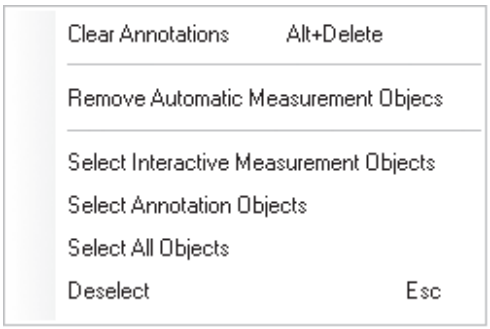
The status bar at the bottom shows the following information (from left to right): pixel coordinates of the mouse cursor with channel intensities, color mode, bit depth, and picture size in pixels.



Working with the Annotation layer

Annotation layer

This button toggles viewing of annotations. Right-clicking the button displays the following context menu:



- **Clear Annotations** will remove all objects from this layer.
- **Remove Automatic Measurement objects** will remove only objects created by automatical measurement. Other objects remain untouched.
- **Select Interactive Measurement objects** will select all measurement objects created by user.
- **Select Annotation Objects** will select all objects that are not the measurement ones.
- **Select All Objects** and **Deselect** will select / unselect all objects stored in this layer.

This button toggles viewing of the measurement objects of the interactive and automatic measurements.

Working with Binary layer

Working with binary images

This button switches to a view of the binary image. It can be edited by hand using **Binary editor** command from the **Binary** menu, or by pressing the **Tab** key.

The Binary layer can be displayed together with the color layer using the **Overlay mode**. The appearance of the binary layer can be modified by right clicking the **Overlay Mode** icon.

This button switches to a view of the color image. When working with RGB image, this layer consist of 3 separate components (Red, Green, Blue). When working with multichannel images, there can be a lot more channels. You can view all these channels mixed together or you can work with (process) only one single channel. To choose it, click on the tab located in the bottom left corner of the document window.

The selected component is marked by color underlining and the pane moves optically to front. (**Red** is selected in the image.) To edit the name or color of component, click on the tab by right mouse button and choose the command from pop-up menu. Custom number of components can be selected while holding the **Ctrl** key down.

Working with Documents

When working with documents it is possible to work with multiple images opened at the same time. If an image has been opened and another one is opened next, the original doesn't close and remains loaded in memory. The Multi-Document Window Environment ensures that every document is opened to a single window that contains the most used controls affecting the image appereance (Layers, LUTs, Zoom etc.).

Opening Saved Files

NIS-Elements offers several ways to open an image file:

Open dialog box

Using the Open dialog-box

To invoke the Open dialog box, you have the following options:

- Select **File** menu - **Open** command...
- Press **Ctrl** + **F12** keys simultaneously...
- Press the **Open** button located on the main NIS-Elements toolbar (left side of the application window)...

Organizer

Using the Organizer

An image can be opened by double clicking its filename within the Organizer layout. More information can be found in the Organizer chapter.

Recent files list

Using the Recent files list

You can quickly access the last opened images using the **Recent files** submenu from the **File** menu. The number of listed files can be changed by user in the General Options window (double click on NIS-Elements caption to display it).

Open next command

Using the Open next/previous/first/last command

These commands enable you to continuously open the next/previous images from a defined directory or database table. This is useful when editing multiple files placed in one directory. The commands are located in the **Open/Save Next** submenu of the **File** menu. You can define sorting, filtering and other features in the Open Next settings dialog box.

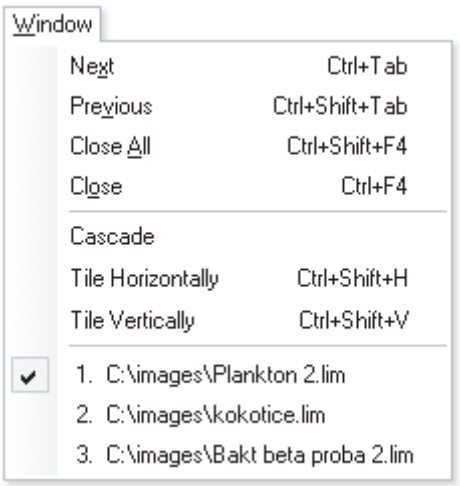
Windows explorer

Straight from Windows explorer

During installation, NIS-Elements creates file associations to files that are considered a native format for storing images (JPEG2000, ND2). The JPEG2000 (JP2) and ND2 image files can be then opened in NIS-Elements just by double clicking their names in Windows explorer.

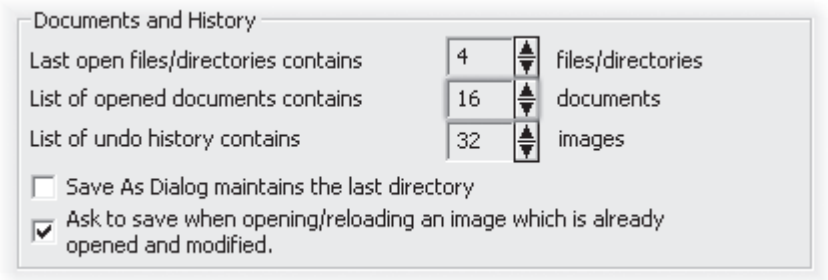
Switching between loaded documents

The commands for managing the opened images are grouped in the **Window** menu. The presently opened files are listed in its bottom part. The currently displayed image is indicated by a check mark on the left side. To change the current image, simply click the window with the desired document. If it isn't possible because of many opened windows overlapping the one you would like to view, select it from the list or use the **Next** or the **Previous** command (represented by **Ctrl + Tab** and **Ctrl + Shift + Tab** shortcuts). Another possibility is to arrange document windows automatically using the **Tile horizontally** or **Tile vertically** commands. This will change the size and position of all opened documents and these will be aligned like tiles in selected direction.



Amount of opened images at one time

In NIS-Elements, it is possible to set the maximum number of images opened at one time. This is done in the General Options dialog box that is displayed after double clicking the NIS-Elements caption.


Documents and History
Last open files/directories contains 4 files/directories
List of opened documents contains 16 documents
List of undo history contains 32 images
Save As Dialog maintains the last directory
Ask to save when opening/reloading an image which is already opened and modified.

The amount is set in the **List of opened documents contains** *n* **documents** edit box. If you set this parameter to 1, NIS-Elements will behave like a single document interface.

Closing documents

The currently displayed image can be quickly closed by pressing the **close window** button, located in the upper right corner of the NIS-Elements document window. (The image displayed below shows the close button of a maximized document.)

The image can be also closed by invoking the **Close** command from the **Window** menu or by pressing the **Ctrl+F4** shortcut. If you want to close all images use the **Close all** command from the same menu, or the **Ctrl+Shift+F4** key shortcut. If you try to close an image that has been changed, NIS-Elements will display a confirmation dialog box, offering to save changes.

Supported image formats

Image Formats

NIS-Elements supports the following number of standard file formats. In addition, NIS-Elements uses its own image file formats (JP2, ND2) to fulfill specific application requirements.

JPEG2000 Format (JP2)

An advanced format with optional compression rates. Image calibration, text descriptions, and other meta-data can be saved together with the image in this format.

ND2 Format (ND2)

This is the special format for storing sequences of images acquired during ND experiments. It contains various information about the hardware settings and the experiment conditions and settings.

Joint Photo Expert Group Format (JFF, JPG, JTF)

Standard JPEG files (JPEG File Interchange Format, Progressive JPEG, JPEG Tagged Interchange Format) used in many image processing applications.

Tagged Image File Format (TIFF)

Developed by Aldus and Microsoft to promote the use of desktop scanners. Used for compatibility with other applications. This format includes information about author, sample, subject, and calibration. Because TIFF files do not have a single way to store image data, there are many versions of TIFF. The system supports the most common TIFF modalities.

CompuServe Graphic Interchange Format (GIF)

This is a file format commonly used on the Internet. It uses a lossless compression and stores images in 8-bit color scheme. GIF supports single-color transparency and animation. GIF does not support layers or alpha channels.

Portable Network Graphics Format (PNG)

This is a replacement for the GIF format. It is a full-featured (non-LZW) compressed format intended for a widespread use without any legal restraints. NIS-Elements does not support the interlaced version of this format.

Windows Bitmap (BMP)
This is the standard Windows file format. This format does not include additional image description information such as author, sample, subject or calibration.

LIM Format (LIM)
Developed for the needs of laboratory image analysis package. Nowadays, all its features (and more) are provided by the JPEG 2000 format.

ICS/IDS image sequence
ICS/IDS sequences are generated by some microscopes and consist of two files: the ICS file with information about the sequence; the IDS file containing the image data. The ICS file must be stored in the same directory together with the IDS file.

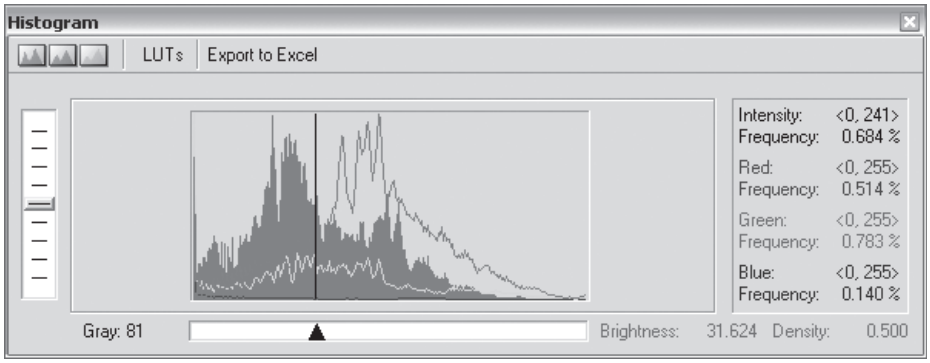
Image Analysis

Histogram and Look Up Tables

Histogram basics

Histogram Window

Histogram is a graphic representation of number of pixels (Y axis) in the image versus their pixel values (X axis). The pixel values run from 0 displayed as black to 255 - 65535 (depending on the image bit depth) displayed as white. In case of grayscale images a vertical bar is plotted for each pixel value showing the number of pixels in the image with that value. Considering color images, such graph is displayed for every color component.



Histogram controls

The picture above shows a standalone histogram window. When this window is docked, it shows only the most significant information to spare space.

Histogram controls

- This button switches the histogram view to a mode with three separate graphs, one for each color component.

- This button switches the histogram view to a mode where all colors and intensity curves are displayed in one graph(see the picture).

Normally, the histogram shows data of the original image. If the **LUTs** button is activated, the histogram displays data as if LUTs were applied.

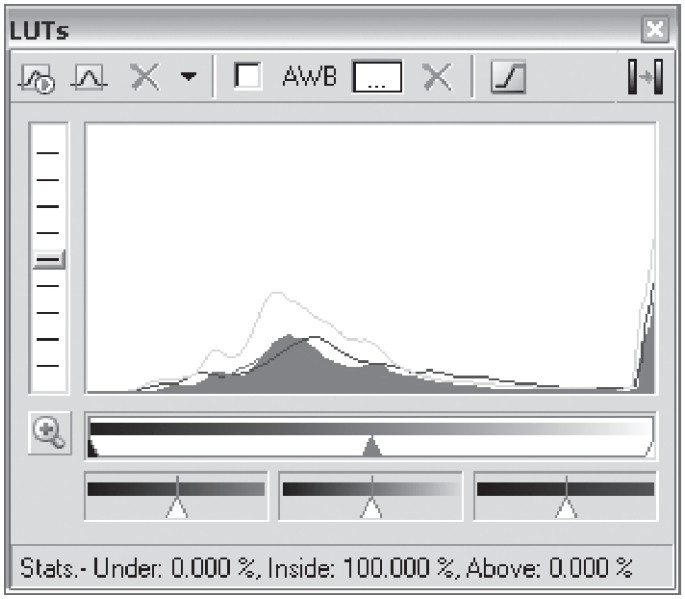
Look Up Tables

LUTs

A look-up table represents a useful tool for color modifications. It takes a value, maps it to a location in a table, and replaces the incoming value with the contents of the table entry. There are 3 modes of LUTs, depending on the image you are processing. Different controls will be available when applying LUTs to monochromatic, RGB, or multichannel image. When LUTs are activated the LUTs button in the upper left corner of the **document window** is highlighted.

LUTs on RGB

LUTs on RGB images



The main part of LUTs window is occupied by a histogram view. There are 3 separate curves for each RGB component, and one gray filled curve for the whole image. You can adjust the histogram view by moving the slider on the left part of the window.

The black and white triangular sliders define threshold. All the pixels with values lower than the black slider indicates (left of the slider) will be displayed as pure black. Everything to the right of the white slider (all pixels with higher values) will be displayed as white. The remaining color shades will be composed of the pixels with values between the two sliders with defineable gamma parameter. Gamma is adjustable by moving the gray slider.

- This button extends the sliders and displays the histogram as if LUTs was applied. The range of pixel values currently displayed becomes indicated in the top right corner.

Three color bars with sliders (displayed underneath the histogram) are representing RGB components. The slider movement affects the brightness of each component.

Auto scale LUTs

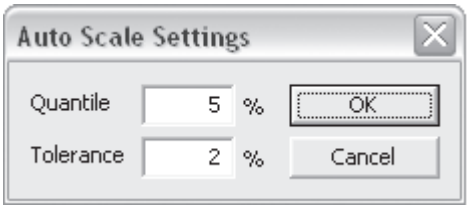
Auto scale

The auto scale mode automatically sets the white slider and gamma parameters to get the best image view (what is „best“ is determined by a universal algorithm, that may differ from your needs). If you select the **Use Black Level** parameter from the pop-up menu, the black slider will be affected too.

It is possible to apply the Auto scale procedure only once by pressing the button, or to run it permamently (on the live image) by clicking the **Keep Auto Scale** button. When you turn the Keep Auto Scale off, the settings remain as if the Auto Scale button was pressed once. If you want to discard all LUTs settings, press the red cross button, located next to the Auto scale button.

Settings

Press the arrow next to the Auto Scale button, a pop-up menu will appear. Invoke the **Settings** command.



Quantile (0-10%) - this value determines how many of all pixels of the picture are left outside the sliders when LUT is applied.

Tolerance (0-10%) - if the threshold decreases within the set Tolerance, LUT remains unchanged and is not re-counted. When the change exceeds the Tolerance, LUT is recounted. This reduces number of LUT calculations when the camera produces picture noise.

White balance

AWB

AWB (Auto White Balance) mode adjusts the image to get the color neutral white. Similarly as above, it can be used once, or permanently on the live image by clicking into the **Keep Auto White Balance** checkbox left of the AWB button. When you uncheck the box, the settings remains as if the AWB button was pressed once.

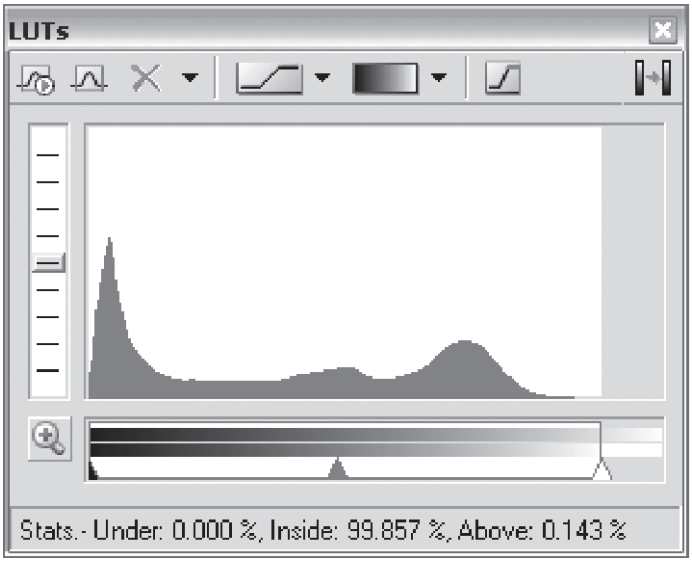
If you know which undertone your white has, you can select this color by the color picker that appears after pressing the **...** button on the right of the AWB button. And again, all changes are discarded using the red cross button.

- The color overexposed button. When this button is activated, all pixels whose values reach maximum will be highlighted.

- Press this button to apply the LUTs settings to the color image data. Until you press this button, no changes are made to the image data.

LUTs on mono images

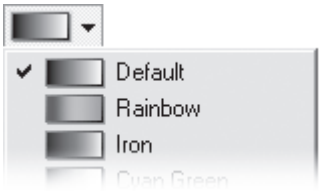
LUTs on Monochromatic images



All things mentioned above are also valid for monochromatic images. When adjusting a monochromatic image, you don‘t have AWB function available and the whole image is controled using only one bar with sliders. On the other hand, you can set more modes of displaying, including colorizing the image using predefined color schemes.

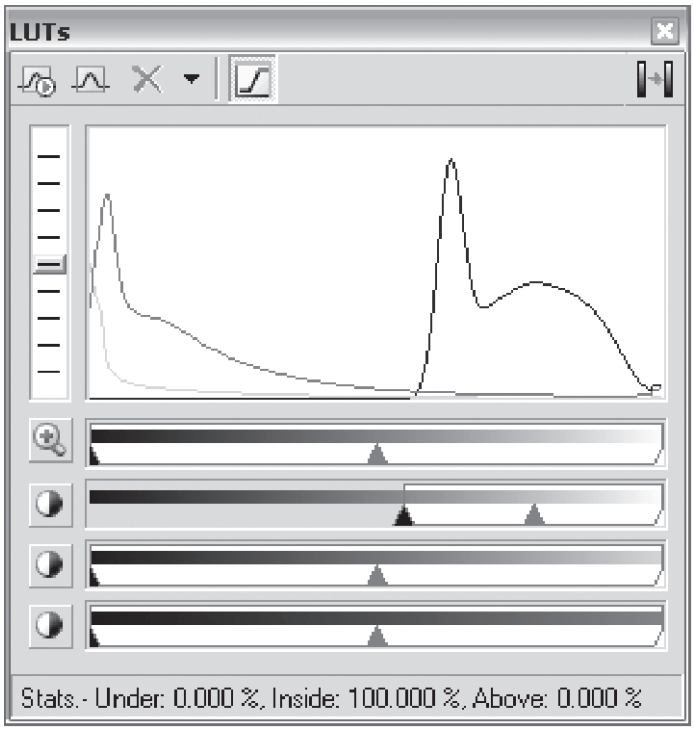
This pop-up menu is used for pickng the mode of mapping. You can select from **Contrast** or **Window** modes. When **Contrast** is selected, all pixels with values higher than the white slider will be set to white. But when **Window** is active all pixels with value higher than the white slider will be set back to black.

This button indicates the selected color table and displays this pop-up menu. There you can select the color scheme, in which you want to display your image. Try a few of them to see which highlights most of the details you want to see.



LUTs on multichannel images

LUTs on Multichannel images



In case of multichannel image the histogram shows different color curves - each color for one component (channel). You can adjust any channel you want using the corresponding bar placed at the bottom of the LUTs window.

- A single channel can be adjusted automatically using this **Auto scale** button.

Look-up tables are useful for equalizing images. For example, if the image is very dark (which usually happens with quantitative cameras), you can restrict the view to display just the low pixel intensities. Hidden details become more apparent.

Binary Editor

Binary Layer

The binary layer, as a result of thresholding, can be modified by hand using the binary layer editor. It is a built-in application providing various drawing tools and morphology commands. Go for the **Binary Editor** command in the **Binary** menu or press the **Tab** key. New controls appear on main application toolbars:

Controls (horizontal tool bar)

- Reverses the changes made to the binary image. **<U>**

- Clears the binary image (fills the entire image with the „background“ color). **<R>**

- Loads the image previously saved by the **Save** button. **<L>**

- Temporarily saves the current binary image. It can be loaded anytime before the binary editor is closed by the **Load** button. **<S>**

- Displays this help page.

- Stores the changes and quits the editor. **<ESC>** or **<TAB>**

The other buttons of the vertical tool bar are simplified versions of mathematical morphology functions. Please see the **Mathematical Morphology Basics** *chapter for more details.*

- Dilate

- Erode

- Close

- Open

- Separate Objects

- Clean

- Fill Holes

Drawing tools (vertical tool bar)

The binary image can be modified using various drawing tools. Although the way of use of some tools differs, there are some general principles:

- Make sure you are in the right drawing mode (drawing background/foreground)
- Any object that has not been completed yet can be canceled by pressing **Esc**.
- The polygon-like shapes are drawn by clicks of the left mouse button. The right button finishes the shape.
- The auto-drawing tools (threshold, auto detect) have a changeable parameter. It can be modified by +/- keys or by mouse wheel.
- The scene can be magnified by the UP/DOWN arrows when mouse wheel serves another purposes.
- The right mouse button drags the image when magnified.
- A line width can be set in the upper left corner.
- Hints are displayed below the horizontal tool bar.

Drawing tools

- Switches between the foreground and the background editing mode. **<Ctrl + SPACE>**

- The Hand tool. Serves for moving the image when magnified. **<Ctrl+W>**

- Bezier hollow tool. The object is defined by placing points on its perimeter. The lines connecting those points can vary from straight lines to bezier curves. (Use +,- keys to adjust them). To finish creation press the right mouse button. **<Ctrl+F11>**

- Bezier fill tool. It equals the Bezier hollow tool, but the resulting object is filled. **<Ctrl+F12>**

- Draws a filled polygon. While holding the left mouse button down, you are in the free hand mode. When you release it, each click defines a corner of the polygon. The polygon is enclosed and filled by pressing the right mouse button. **<F4>**

- Draws a polygon. It equals the Filled polygon tool, but the resulting object is not filled. **<F3>**

- Draws a filled circle. Click to determine the center and define the perimeter holding the left mouse button down. **<F8>**

- Draws a circle. Click to determine the center and define the perimeter holding the left mouse button down. **<F7>**

- Draws a filled rectangle. **<F10>**

- Draws a rectangle. **<F9>**

- Draws a filled moveable circle/ellipse. If you grab the ellipse near the center, you can move it. If you grab it near the border, the nearest semi-axis is being modified. When holding down either the SHIFT or the CTRL key, both semi-axes change equally (forming a circle). **<F12>**

- Draws an ellipse. **<F12>**

- Drawing by hand. **<F1>**

- Draws a straight line. **<F2>**

- Draws a polyline. **<F5>**

- The Auto Detect filled tool. Detect hollows using threshold techniques. Click to the image to place the probe, the detected area is drawn. You can adjust the thresholding range by using the mouse wheel or by pressing the +, - keys. **<Ctrl+B>**

Auto white balance tool

- Threshold tool. Click into the image to place the probe (or more of them) to define the initial color level for thresholding. Pres +,- keys or use the mouse wheel to change the thresholding range. **<J>**

- Fill an enclosed shape. **<F6>**

- Area of interest. Entities outside the selected region will be cleared.

- Text tool. Displays dialog box for defining text parameters.

- **Commands** tool. Displays a pop up menu offering user some additional commands:

Commands

- The Auto Detect hollow tool. Detect hollows using threshold techniques. Click to the image to place the probe, the hollow is drawn. You can adjust the thresholding range by using the mouse button or by pressing the +, - keys.

- Draws rectangularly crossed lines.

- Draws a rose. Click to the image to define the center, than drag the mouse to set the length of it‘s arms.

- Places a marker to the image. Simply click into image...

- Selects binary objects.

- The Connect tool. Draws a line(s) from the point you‘ve clicked on, to the nearest object(s).

- Displays a grid. Visible only when using magnification 400% and higher.

- Hides the binary image and displays the just the color image.

- Inverts the binary image. Foreground becomes background and vice versa.

The last two buttons perform simplified versions of mathematical morphology functions. Please see the **Mathematical Morphology Basics** *chapter for more details.*

- Smooth

- Contour

Tools are either applied in a „draw“ (foreground) or „erase“ (background) mode. Some tools (like threshold or bezier) have editable parameters. Tips how to use the tools are displayed in the vertical toolbar.

Measurement

Measurement is an advanced and complex image analysis technique. It starts with calibration of the NIS-Elements system which is followed by a quantitative evaluation of the image. Image analysis is finalized with data examination and presentation.

Calibrating system

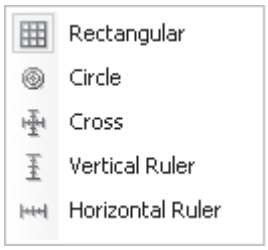
Calibration

Calibration is of a crucial importance for the measurement. It assigns real sizes to objects, so they can be compared to each other. A correct calibration has to be made before the measurement begins. See the **Optical configurations** section explaining how to calibrate the NIS-Elements system.

Rough measurement

Rough Measurement

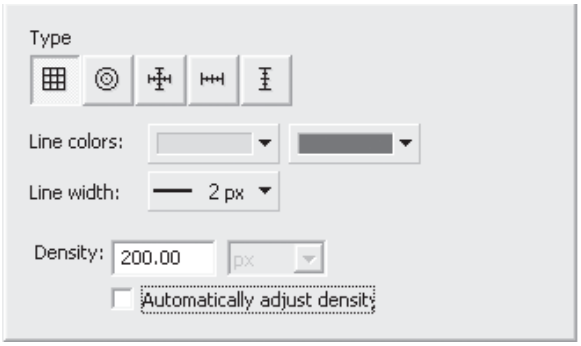
Quick and approximate measurements can be performed with the graticules. They behave like adjustable floating rulers. The user can simply align it with the measured object and read the distance (e.g. the diameter). To activate a graticule, press the Graticules button placed in the upper-right corner of the **document window**) The type of the graticule ruler is indicated by a picture on this button. The types are: *Rectangular*, *Circle*, *Cross*, *Vertical Ruler*, and *Horizontal Ruler*.



To change the graticule type, click on the graticules button with the right mouse button and select the appropriate item from the context menu. The menu also contains two commands for placing the graticules ruler into **binary image layer** and vice versa. The command *Move Graticules to Center* aligns the center of the ruler with the current center of screen. The last command - *Graticules properties* - displays the following dialog box:

Measurement options

In this dialog box all parameters (shape, color, line width, density of lines) can be adjusted. The density value - the closest distance between two line intersections of the graticule - can be set in pixels (in case of uncalibrated image) or in selected units. The density can be also adjusted automatically according to the current zoom factor.

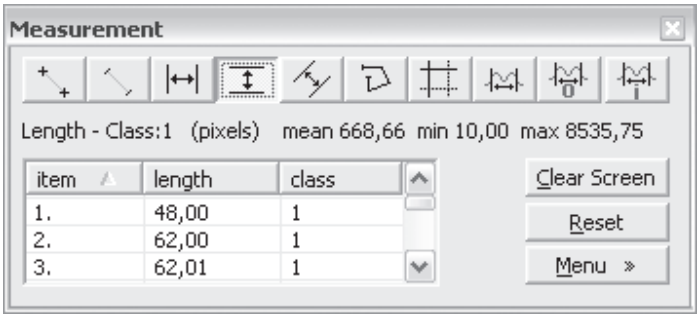


Interactive measurement

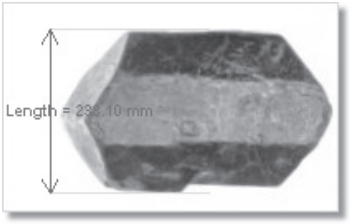
Interactive measurement

The interactive measurement procedure means that the user measures directly on the screen and gets precise outcome in selected units. The measurement is performed by drawing e.g. two parallel lines, aligned with borders of the measured object. This is the way of defining the boundaries of the object in the image. The real size of the object is then counted using calibration constant.

The interactive measurement can be performed on both color and binary images. First, select the feature you want to measure. You can choose from: *Taxonomy*, *Counts*, *Length*, *Semiaxes*, *Area*, *Angle* or *Profile*. The selection is done by picking one of those commands from the Measurement menu. For example, to measure the distance between two objects in the image, select the *Length* command and the following dialog box appears:



The buttons in the upper part of the window represent different measuring tools. Detailed info about any of measuring tool can be found in the Command Reference section of the help. The following example illustrates how to measure the size of a crystal on its binary image:



Select the interactive tool *Horizontal parallel lines*. Place the first line to its position by clicking into the image. Hold the left mouse button during positioning, after you release it, the line is positioned. Repeat it for the second line. When finished, an arrow is drawn between those lines, joined by the value of the measurement. A record with the measurement type and measured value is added to the table in the Length dialog box (pictured above).

Objects and field measurement

Object and Field measurements

When starting measurements, it is important to decide whether a field or an object should be measured. Generally, NIS-Elements distinguishes two kinds of measurements, object measurements, or field measurements.

Object Measurements

Groups of neighbouring pixels of the binary layer are called objects. Object measurements provide user with specific object data such as Length, Area, CentreX/Y (the X,Y position of the central pixel of an object), etc.

Field Measurements

Field measurements produce information regarding the whole measurement frame area such as Area Fraction, Mean Brightness, Density Variation, etc.

Using measurement frame

Measurement frame

The Measurement frame has different meanings for object and field measurements. In the object measurements, the particles touching the left and bottom lines are excluded from the statistics of objects, whereas particles touching the top and right lines are included. In case of the field measurements, the measurement frame defines the rectangular area to which measurements of features are restricted. To set up a measuring frame, choose the Measurement Frame item from the Measure menu and type the co-ordinates of the top-left and bottom-right corners or drag the measurement frame rectangle by the left mouse buton.

Image types needed for measurement

Images involved in measurement.

Measurements can be performed on both types of images:

- color images
- binary images

For instance, intensity or typical hue measurements are carried out on color images. Binary image is used for shape and size measurements (area, perimeter, surface fraction). Mask image is used when you wish to restrict the measurements to a non-rectangular area - referred to as an area of interest. The area of interest is defined as an intersection of the measurement frame and a mask image.

Measured data

Statistics and data presentation

NIS-Elements calculates basic statistics - mean value, standard deviation and distribution of all measurement features. NIS-Elements supports *selective* definition of a measurement when you can customize a general measurement by selecting features of interest. Further, it enables *real time*, and *interactive* data observation: when the user defines fields for statistics evaluation. NIS-Elements provides two ways of graphic data presentation: direct, immediate graphic presentation of a feature distribution in the NIS-Elements environment, or via the clipboard, for graphic presentation of the data. See also the Object Data and Field Data dialog boxes.

Densitometric and brightness measurement

Densitometric and brightness measurements

NIS-Elements can also perform densitometric and brightness measurements on the red, green and blue components. NIS-Elements transforms the camera output, which is strongly dependent on the camera setup. Therefore any intensity quantification of images needs precise calibration. Generally speaking, light intensity measurement is performed in any case, but there are two arrangements of special interest. If we measure the light absorption (the transmission through an absorbing sample) it is referred to as densitometric measurement. In the other case the measurement of the reflected (reflectance measurement on coal samples) or emitted light (fluorescence) is referred to as brightness (intensity) measurement. The term „optical density“ requires an explanation:

We denote the light intensity incident on some absorbing media by I_0 (DNA staining of cell nuclei) and the transmitted light by I. We can then define the transmittance T as the ratio I/I_0 and the optical density (O.D.) as negative logarithm (base 10) of the transmittance. The definition of optical density is correct for one pixel. For object or field measurements it is necessary to define the integral (optical) density, as the sum of O.D. values over the object/phase. The optical density is also referred to as density in this manual. Monochrome cameras output electric video signal which depends on the intensity of incident light (on the surface of CCD chip). This dependence is characterized by the gamma coefficient of the camera (some cameras have fixed gamma coefficient, others allow gamma adjustment usually from 0.45 to 1). Because the camera also allows setting the offset and gain independently of gamma coefficient, gray pixel values cannot be considered as absolute values. It is even more complex issue because of the grabber setting parameters. The formula characterizing the gray value on light intensity dependence is as follows:

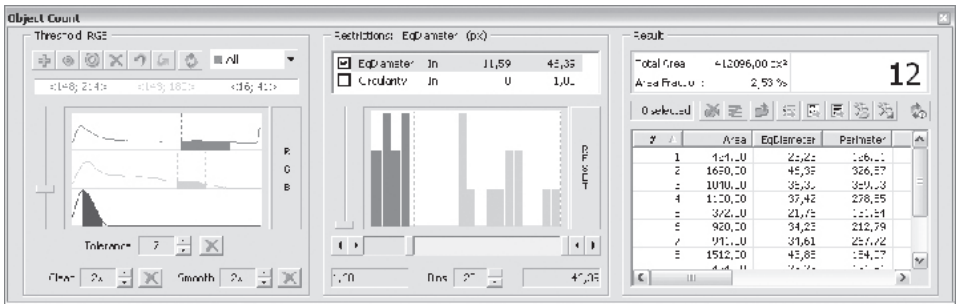
$$grayvalue = offsetcoef + gaincoef * I^{\gamma}$$

NIS-Elements allows performing two types of calibration: density and brightness (intensity) calibration. At least two points are necessary to determine the calibration curve. This is the simplest case: gamma is known, and zero intensity (no incident light) is set to zero gray value, then only one standard is necessary for calibration. NIS-Elements also supports calibration using 3 standards (one of them could be a shut camera with zero intensity). In this case the gamma coefficient is calculated. NIS-Elements even offers a general calibration scheme, using from 4 to 8 standards. In this case the calibration may deviate from the simple brightness (intensity) or density quantification and may be linked, e.g. to weight (biological mass).

Object Count

Object count window

This control window combines important image analyzis tools together and enables user to threshold, measure the image, and export the measured data to a file in a smooth way. Object count can be performed even on Live image.



Thresholding

Thresholding the image

Threshold limits have to be defined by choosing reference points in the image. Select one of the following tools:

- **1 point threshold tool.**
- **3 points circle threshold too.l**
- **6 points circle threshold tool.**

Click inside the image to define the objects. The system will detect similar parts of the image and highlight it by color edges and fill. The threshold limits are indicated in the below histogram and can be further modified (dragging the limits by mouse). The channel of which the histogram is displayed can be selected from the rightmost combobox (All(RGB), single channel, Intensity).

Threshold modifications

Use the following tools to modify the threshold:

- **Reset threshold** deletes all the Object Count (OC) objects.
- **Undo threshold** returns the OC layer one step back.
- **Redo threshold** returns the OC layer one step forward.
- **Rethreshold image** updates the OC layer according to the most recent threshold settings.

Tolerance
Threshold limits are always stretched by the tolerance number.

Clean
To remove very small OC objects that should not be included in the result, the binary **Clean** operation can be applied to the OC layer one or more times.

Smooth
This binary transformation smothes the edges of the OC objects.

Measurement restrictions

Applying measurement restrictions
The number of objects that are included in the results table can be restricted by defining some limiting features.

- Right click to the restrictions list field to select a limiting feature. **Area**, **EqDiameter**,**Perimeter**,**Length**,**Width**,**MaxFeret**,**MinFeret**,**Circularity**,**Elongation**,**MeanIntensity**,**SumIntensity**fetures are available.
- The nearby checkbox indicates whether it is applied or not. If applied, the histogram of the feature is color (otherwise it is gray).
- Select the restriction feature you would like to define.
- Move the sliders below the histogram to set the limits. The limit values are indicated next to the feature name.

Reset
The Reset button discards restriction settings of the currently selected feature.

Bins
This is a number of columns in the histogram.

Measured data

Working with measured data
The following results appears in the results table:

- **Total Area** is the area of the whole image in px^2 or $[\text{calibration units}]^2$.
- **Area Fraction** is the ratio between Total Area and the area of the thresholded objects in %.
- **Number of objects** counts the thresholded objects.
- **Table of results** - every thresholded object is measured and the results are inserted to this table. Select the columns to display/hide from the context menu that appears when you right-click one of the column names.
- **# selected** - number of selected objects is displayed in this box. One ot more objects can be selected by mouse while holding the **Ctrl** key.

The following tools can be used:

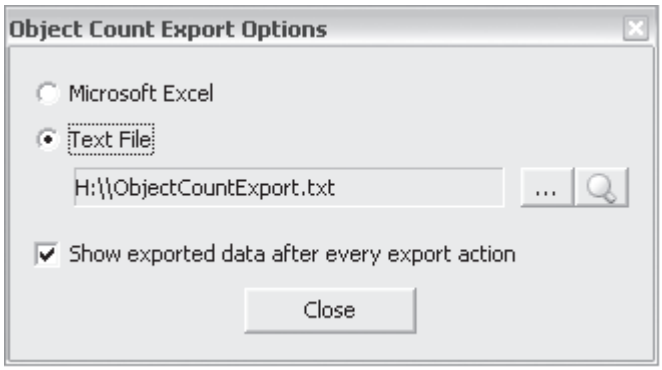
- **Delete selected objects** removes the selected OC objects. The objects can be selected by mouse holding the **Ctrl** key down.
- **Invert selection** deselects the selected OC objects and vice versa.
- **Generate binary** converts the current OC layer to binary layer (the previous binary layer is overwritten).
- **Load OC configuration** loads the saved settings.
- **Save OC configuration** saves the threshold and restrictions settings under a user-defined name.
- **Keep updating count** refreshes the measured results each time the OC layer changes.

Data export

Exporting Measured Data

- **Export options** enables to choose the export format (xls or text file).
- **Export all data** copies the measured data to xls/text file. The data are ordered and there is a header above each measurement.
- **Export raw data** copies raw data to xls/text file.

Once you have the data, they can be exported to a file. Click the Export Options button, the following widnow appears:



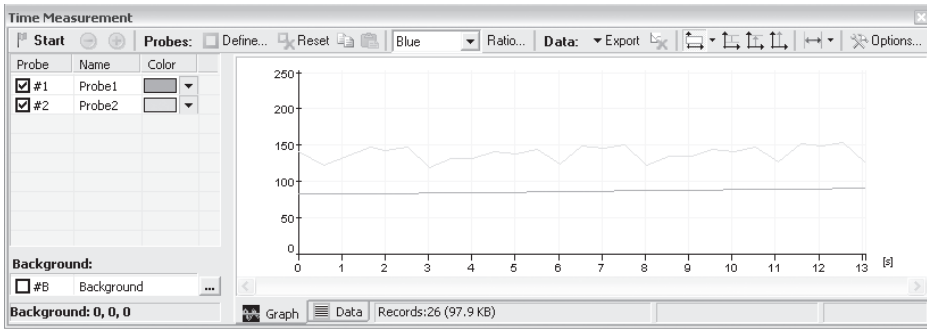
- Select whether to export the data to an Excel file or to a text file.
- The path and the name of the text file can be modified via the Browse button. You can also view the file using the Preview button.
- When the **Show exported data after every export action** box is checked, the system switch to Notepad each time the export to text file is performed.
- The newly exported data are appended to the end of the export file.
- When exporting data to MS Excel, system runs the aplication and inserts the data there.

- When exporting data to text file, the file does not need to be opened - depending on the above checkbox, the system just appends the data to the selected text file.
- Confirm the settings by **OK**
- Press the **Export all data** or **Export raw data** button.

Time Measurement

General overview

The time measurement tool enables to record avarage pixel intensities within defined probes during a time interval. The measurement can be performed on Live camera signal. Before you can start measuring, following actions shall be done: probes definition and the options selection. Go to View>Controls>Time Measurement command to open the Time Measurement control window. All commands and buttons described below relates to this control window.



Defining probes

Probes Definition

To define 1-31 areas (probes) where the measurements will be performed:

- Click the **Define Probes** button.
- The toolbar containing five different tools for creating the probes appears.
- Select one of the tools and create the probes.
- Click the Finish button to confirm the action.

Rectangle

Draws rectangular probes.

Ellipse

Draws elliptical probes.

Freehand

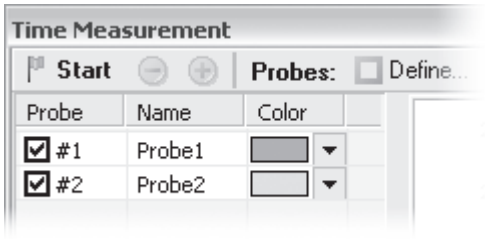
A polygon can be drawn by defining the node points, or draw arbitrary shape holding the left mouse button down. Finish the creation by right click.

Auto Detect

This tool estimates the edges of a probe according to a threshold criterion. The detection parameters can be modified via the mouse wheel, arrow keys and the S key (separate). Please see the NIS-Elements caption for hints.

Use Binary

Objects of the binary layer can be also transformed into probes. If there is more than 31 binary objects, the largest ones will be used.

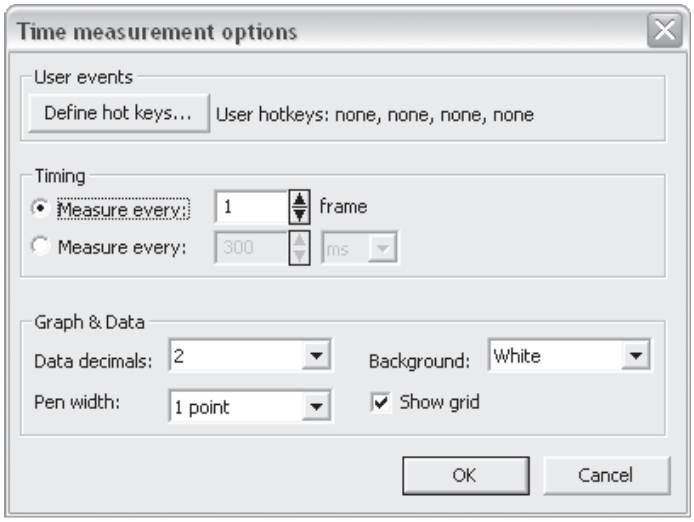


Visibility, names, and colors of the probes can be modified from the control window. As well as the position and rotation angle can also be changed directly in the picture. The probe can be duplicated or copied to another document too. Measurement is performed on the current live image under all visible probes.

Measurement options

Measurement Options

Click the **Options** button. There you can specify the time measurement details in the following sections.



User events

Arbitrary time of some user events can be indicated in the data table or the graph by a marker.

- **Define Hot Keys** - Press this button to define up to 4 hot keys. Select a shortcut key combination and insert a user text. A marker and the user text will be inserted each time the shortcut is used during the time measurement.

Timing

A time interval shall be set to determine how often one partial measurement will be performed.

- The **Measure every N frame** section defines after how many (N) frames one measurement is performed.
- The **Measure every N ms** section defines directly the time interval in miliseconds.

Graph & Data

Some of the schematic properties can be modified:

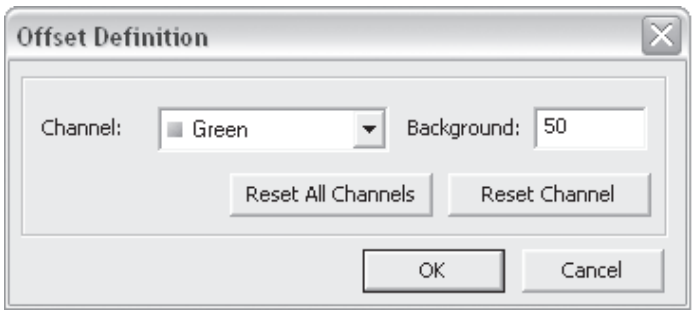
- **Data decimals** - select the precision of the results in the data table.
- **Background** - choose the color of the graph background.
- **Pen width** - defines the graph line width.
- **Show grid** - shows a schematic grid inside the graph.

Background subtraction

Background subtraction

A constant value can be subtracted from every measured value:

- Check the **#B** box of the **Background probe** to extract the value from the area of the background probe.
- Or, you can define a value for each channel in a separate dialog box. Click the nearby **...** button.



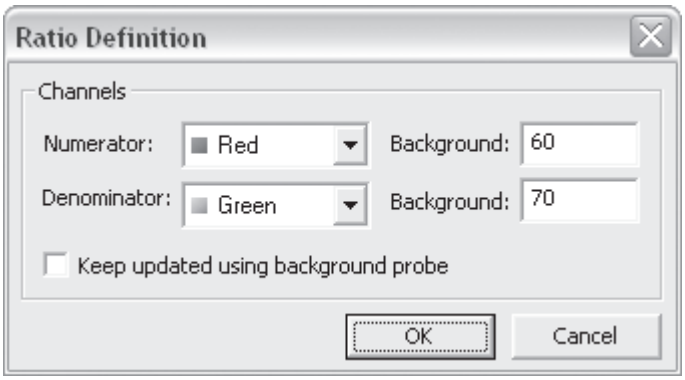
- Select a channel and define the background value, or one value for all (RGB) channels can be input.

Ratio measurement

Ratio measurement

There is a possibility to measure the ratio between two color components of the image. The color components can be set in this section:

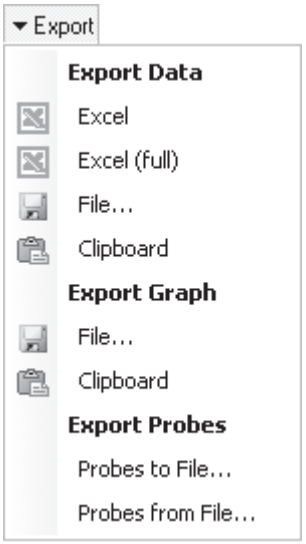
- Click the **Ratio** button on the horizontal tool bar
- The following dialog window appears.
- Select the channels and define the background value to be subtracted.
- The background can be also subtracted using the background probe. Just check the **Keep updating using background probe** box.



Measured data

Measured Data

Once you have set the options and the timing, press the Start button. The measurement runs until the Stop button is pressed. The measured data can be viewed as a graph, table, or exported.



Data export

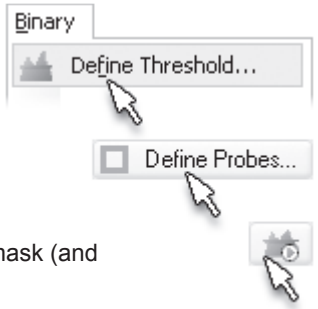
Data could be exported directly to Excel, to text file (the data separator is TAB), or to clipboard. The graph can be exported to a file or to a clipboard in graphical metafile format. The export is done via the **Export** menu or the context menu.

The probes that you have defined can be saved to a file and loaded later. Click the **Probes to file** command to save it to disk in a *.dat file. The **Probes from file** command loads the saved probes and replaces the current ones.

Special Features

Measuring under a dynamic binary mask

The Dynamic Threshold feature can be used to restrict the measured area of the defined probes:



- Define Threshold by the Binary>Define Threshold command.
- Define probes over the Live or Frozen image as described above.
- Click the Enable Dynamic Threshold button.
- Start time measurement by clicking the Start button.
- Only values of that pixels covered with the dynamic binary mask (and placed within the probes) are taken into account.

Graph Autoscale

There are the following buttons affecting the graph view:

- **Autoscale Graph Vertical Fixed** zooms the Y axis within a defined range. Use the **Define Range** command from the context menu to set the limits.

- **Autoscale Graph Vertical** zooms the Y axis to fit the window.

- **Autoscale Graph Vertical from Zero** zooms the Y axis to fit the window and preserves all Y values including the origin visible.

- **Autoscale Graph Vertical OFF** performs no automatic scaling.

- **Autoscale Graph Horizontal** stretches the graph so that the whole X axis or a defined time interval is allways visible. The time interval can be selected from the context menu.

Mathematical Morphology Basics

Overview

The binary image as a result of thresholding often needs to be modified before any measurements are performed. Edges of the objects can be smoothed, holes in the objects filled etc. by using the mathematical morphology commands. "Image Analysis and Mathematical Morphology" by J. Serra (Academic Press, London, 1982) is used as a reference publication for the following overview.

Basic transformations

Basic processes of mathematical morphology are: erosion, dilation, open, close and homotopic transformations.

- **Erosion** -After performing erosion, the objects are shrunk. It means that the inner layer of objects is subtracted. If an object or a narrow cape is thinner than the thickness of the inner layer they disappear from the image.

- **Dilation** - After dilation the objects are enlarged. That means the outer layer of the objects is added. If the distance between two objects is shorter than the thickness of two layers these objects are connected together. If a hole is smaller than the thickness of two layers, it disappears.

- **Open** - In this case erosion is followed by dilation so the size of the objects is not significantly affected. Contours are smoothed, small objects are suppressed and gently connected, particles disconnected.

- **Close** - In this case dilation is followed by erosion so the size of objects is not significantly affected. Contours are smoothed, small holes and small depressions are suppressed. Close objects may be connected together.

Homotopic tranformations

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Homotopic transformations

Transformations that preserve topological relations between objects and holes inside them. Using homotopic transformation, an object with 5 holes should be transformed to another object with 5 holes. Two objects without holes should become also two objects without holes, but probably with a different shape and size. Opening, Closing, Erosion and Dilation are not homotopic transformations. Typical homotopic transformations in NIS-Elements are: Skeletonize, Homotopic Marking and Thickening.

There are other very important transformations of binary images implemented in NIS-Elements binary editor:

Advanced tranformations

- **Clean** - This transformation is also called geodesic opening. First, the image is eroded and small objects disappear. Then, the remaining eroded objects are reconstructed to their original size and shape. The advantage of this algorithm is that small objects disappear but the rest of the image is not affected.
- **Fill Holes** - Fills the holes inside the image. For example, you can detect only boundaries of the objects because objects have rich inner structure with intensities typical for background and boundaries. After filling the holes, boundaries are transformed to closed areas.
- **Contour** - This transformation converts an object into its contours.
- **Smooth** - Smooth transformation affects sharp edges and smooths them.
- **Morpho separate objects**This transformation detects standalone objects that are connected together and detaches them.

Note:

Applying the above mentioned transformations in computerized image analysis brings some limitations due to digitization. In digitized images, information is known only at grid verticals and limiting cut-out of real scene is available. This is the reason why the most frequent terms in binary image processing are: structuring elements, connectivity, grids, problem with border areas.

When speaking about binary image processing, binary image is considered as a set of pixels where values 1 represent objects (white areas) and values 0 represent background (black areas). NIS-Elements system uses a square grid. In this type of grid there are two possibilities of connectivity, the 8-connectivity or the 4-connectivity. The difference between the 8 and 4 connectivities is shown in the example picture. If the 8-connectivity is considered, there is one object in the picture, if the 4-connectivity is considered, there are two single point objects in the picture. NIS-Elements works with the 8-connectivity model, so all points neighboring by the corner belong to one object.

Implementation

Implementation in NIS-Elements

Erosion, dilation, opening and closing are defined by Matrix and Number of iterations. Matrix is also known as the kernel or structuring element. There are the following structuring elements used in NIS-Elements:

The bright pixel in the center or near the center defines the central point of a structuring element.

Erosion

Erosion

Lets assume the 1 and 0 values represent object(1) and background(0) pixels. You can imagine an erosion by a Matrix as the following algorithm:

Move the bright pixel of the Matrix to every point of the image. Each time, look at the neighborhood and make the following decision:

- If there are object(1) pixels in all the positions of the Matrix, set the bright pixel to object(1).
- If there is at least one background(0) pixel in the neighborhood, set the bright pixel to background(0).

The second parameter, the Number of iterations defines how many times the erosion is to be applied.

Dilation

Dilation

You can imagine a dilation by a Matrix as the following algorithm:

Move the bright pixel of the Matrix to every point of the image. Each time, look at the neighborhood and make the following decision:

- If there is at least one object(1) pixel in any position of the Matrix, set the bright pixel to object(1).
- If there are background(0) pixels in all the positions of the Matrix, set the bright pixel to background(0).

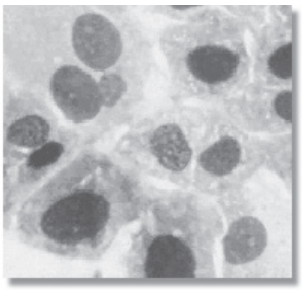
Open & Close

Open and Close

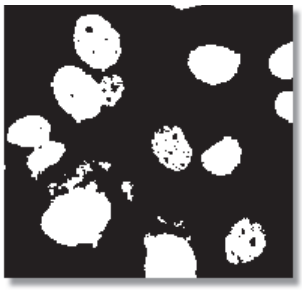
Open is performed by eroding the image and then applying a dilation to the eroded image. On the contrary, Closing is performed as a dilation followed by erosion.

Note:
If the bright point is not in the center (the first and the third example picture above), then applying erosions or dilations by odd number of iterations causes image to shift by 1 pixel. Normally the total image shift would be given by the Number of Iterations (in pixels). NIS-Elements eliminates this shift: it changes the position of the bright point 1 pixel down--rightwards within the structuring element for even operations. For opening and closing it is possible to eliminate this shift totally. However, if you run the erode or dilate processes again and again (e.g. calling the Repeat Last command) using the Matrix with even dimensions and odd number of iterations, then the shift is significant.

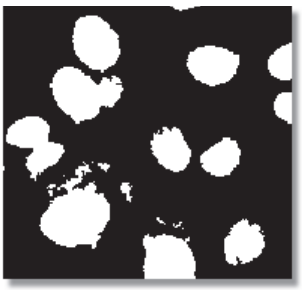
Examples:



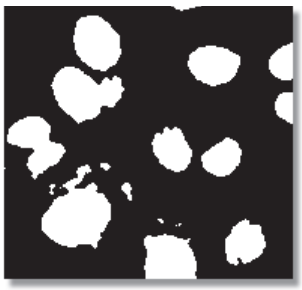
Original Image



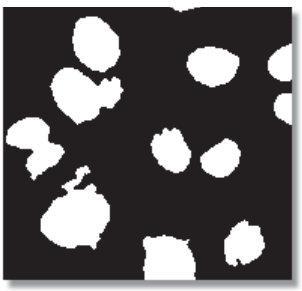
Threshold();



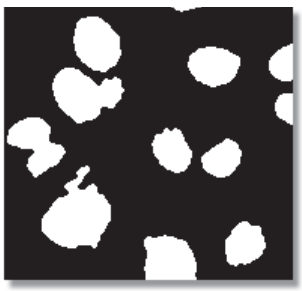
CloseHoles();



SmoothBinary();

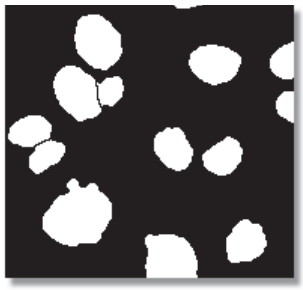


CleanBinary(2, 4);



OpenBinary(1,4); CloseBinary(1,4);

OpenBinary(2,4);



MorphoSeparateObjects(4,1);

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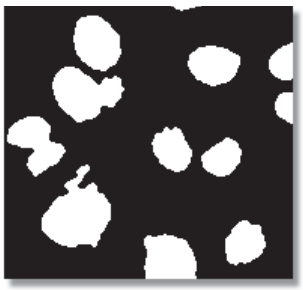
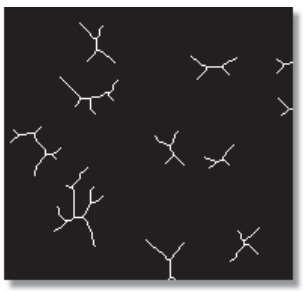


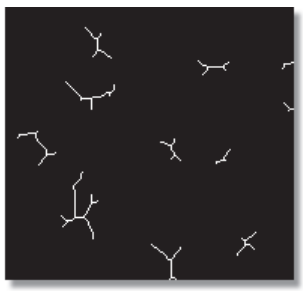
Source

Skeleton

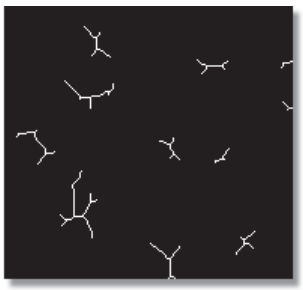
AR



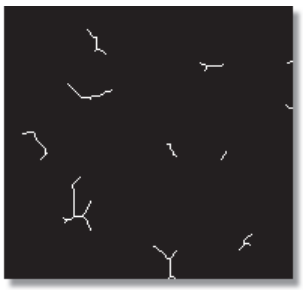
Source



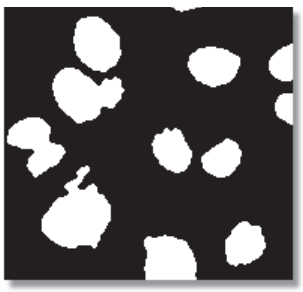
Prunning 3-times



Source

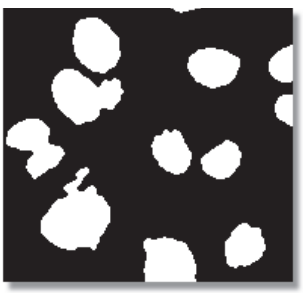


Prunning 2-times

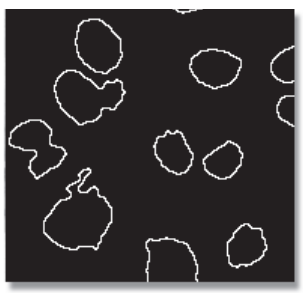


Source

Convex Hull

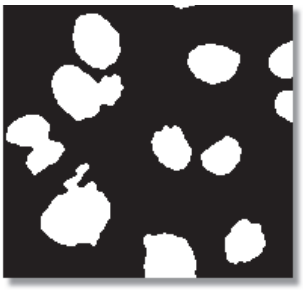


Source

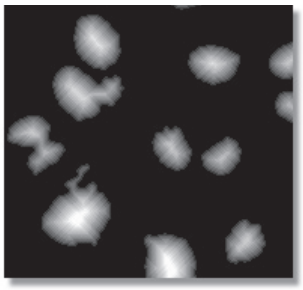


Contour

AR

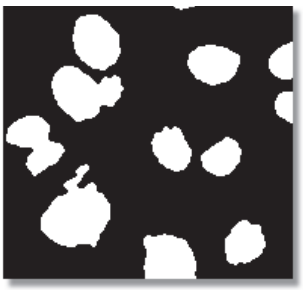


Source



Distance Function

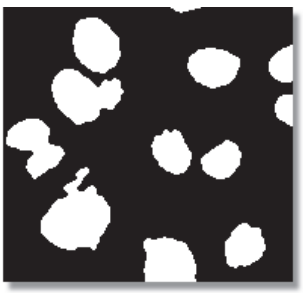
AR



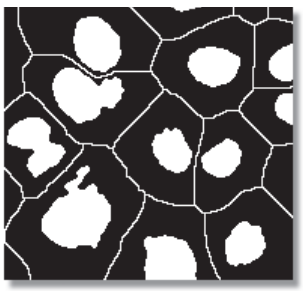
Source

Ultimate Erosion

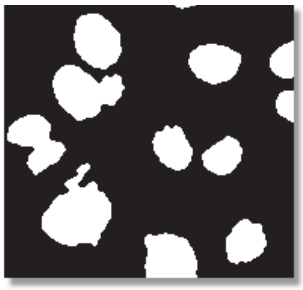
AR



Source

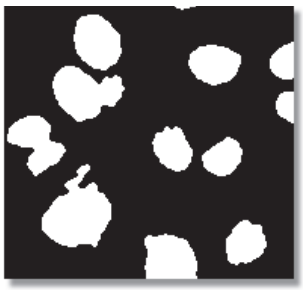


Zones of Influence + Source

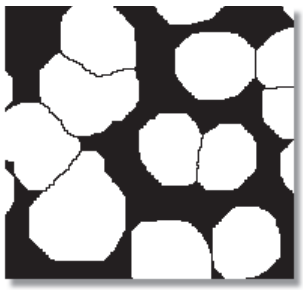


Source

Homotopic Marking



Source



Thickening 9-Times

How to create a simple macro?

Creating macro possibilities

An executable sequence of NIS-Elements commands saved to a single file can make the work very efficient. The macro, as the file is called, can be created either by recording the actions being performed, by writing the functions straight inside the macro editor, or by modifying the history of commands which have been executed recently (the history is recorded automatically during the work).

Recording macro

Recording a macro

The fastest way to create a macro is to record it. To do so, start the NIS-Elements macro recorder by checking the Record item from the Macro menu, and then perform the series of actions you would like to record. NIS-Elements records all actions until you invoke the Stop Recorder item in the Macro menu.

Editing macro

Writing/Editing a Macro

There is another tool for creating and editing macros designed for complex tasks. NIS-Elements provides a macro editor with its own toolbar. Besides the common commands such as Copy, Paste, Cut, Save, Print, and Undo it also supports the Paste Command (from the list of all available functions) command, a semi-automatic macro creating procedure (Insert Commands from History command), and the Help with detailed functions description.

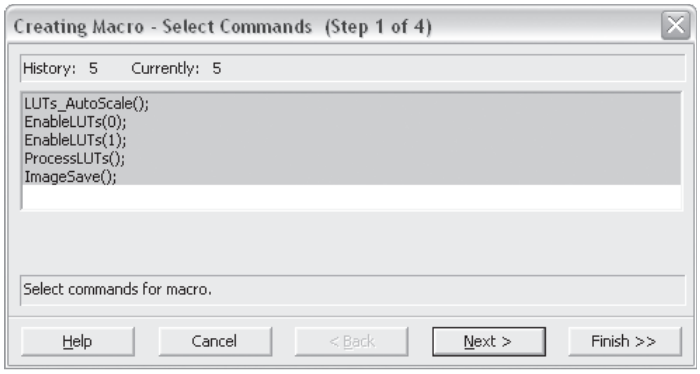
Macro from command history

Creating a Macro from History

You can create a macro using the list of commands you have performed recently. To create the macro in this way choose the Macro menu > History. The Command History dialog box appears and by pushing the Create Macro button, the „Create Macro“ wizard appears. You can also call this wizard from within the macro editor by pushing the Insert Commands from History button.

Selecting commands

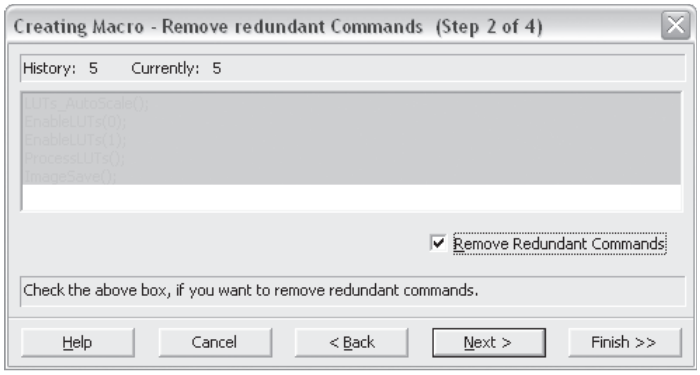
1. Creating Sequence - Select Commands (Step 1 of 4)



- **History** - number of functions performed in the NIS-Elements session.
- **Currently** - number of currently selected functions.

Redundant commands removal

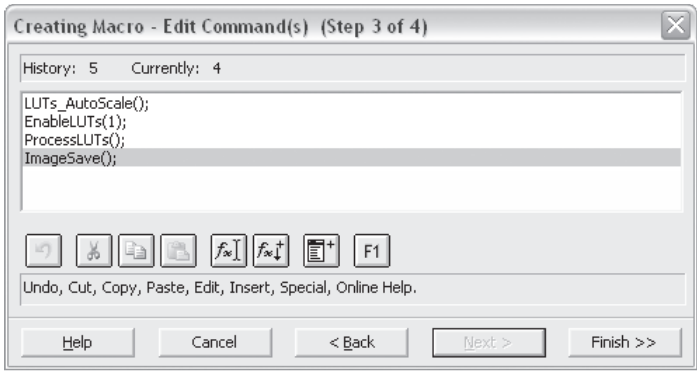
2. Creating Sequence - Remove Redundant Commands (Step 2 of 4)


Creating Macro - Remove redundant Commands (Step 2 of 4)
History: 5 Currently: 5
Remove Redundant Commands
Check the above box, if you want to remove redundant commands.
Help
Cancel
< Back
Next >
Finish >>

Remove redundant commands - the sequence of commands that make no image changes is automatically removed. Usually, it concerns commands that toggles the image between two states and were used several times in a row. It can be for example the ShowAnnotations(), ShowProbe(), or EnableLUTs() command. No functionality is lost when only the last command is kept.

Editing single commands

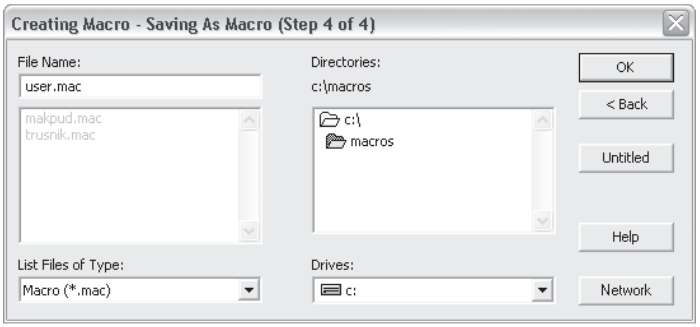
3. Creating Sequence - Edit Single Command (Step 3 of 4)



- Reverses changes that you made within this dialog window such as cutting, copying, pasting or inserting functions.
- Removes selected function(s) and puts it into the clipboard.
- Copies selected function(s) into the clipboard.
- Pastes function(s) from the clipboard before the currently selected function.
- Enables editing the selected function.
- Inserts a command from the list of all available functions. Select the function you want to paste and press the OK button.
- Inserts a special command. The dialog window that appears includes the most frequently performed actions, such as Run Macro from File, Pause Macro, etc. Select the action you would like to insert and press the Insert button.

Saving macro

4. Creating Sequence - Saving As Macro (Step 4 of 4)



Saving a Macro

If you create a macro by recording subsequent actions, you can save a macro using the Save or the Save As item in the Macro menu. To save a macro during editing it, press the Save button in the macro editor.

Running macro

Running a Macro

NIS-Elements provides several ways to run a macro.

- You can run the current macro loaded to NIS-Elements when choosing the **Run** command from the Macro menu or by pressing **F4**.
- You can run a macro that is opened by the Open command from the Macro menu. Then press **F4**.
- You can run a macro directly by pressing a special **hotkey** combination (Ctrl+Alt+1,2,3 ... 9) that you have assigned to it by the Options command of the Macro menu.
- You can run a macro at the beginning of the NIS-Elements session, by assigning a **StartUp** flag to the macro by the Options command of the Macro menu.
- You can run a macro directly by using the **Run From** command of the Macro menu.
- You can run a macro by clicking the **Tool bar button** that you have assigned to it by the „View > Customize Toolbar > Setup“ command.

Pausing macro

Breaking and Pausing a Macro

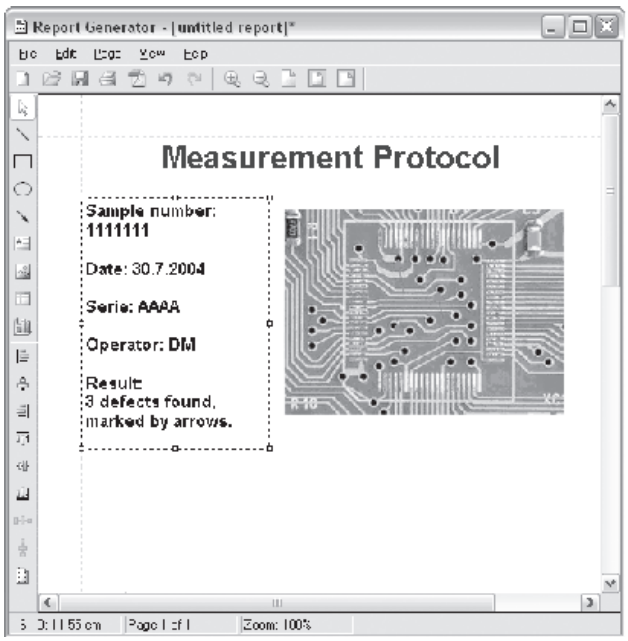
If you want to break macro execution, press Ctrl+Break, (Ctrl+Pause) or the toolbar button with the StopMacro function. If you want to pause macro, call PauseMacro function.

Creating Reports

Useful reporting tool

Report Generator enables the user to create customized reports containing images, database descriptions, measured data, user texts, and graphics. PDF format files can be created directly from NIS-Elements.

When you invoke the **File > Report > New Blank Report** command, an empty Report Generator appears. It provides the following tools for working with objects:

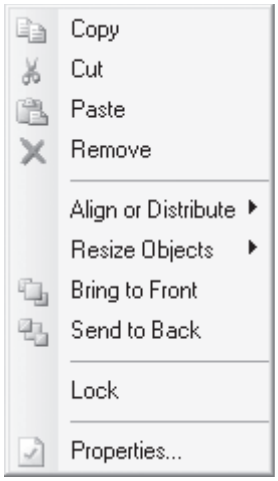


Generator buttons

- - Draw line...
- - Draw rectangle...
- - Draw ellipse...
- - Draw Arrow...*
- - Insert text field...**
- - Insert image from file...
- - Insert Table
- - Insert graph
- - Aligns the selected objects to the position idicated by the line (left)
- - Aligns the selected objects to center
- - Distributes the objects regularly in specified direction
- - Determines whether to apply the align commands relatively to the whole page or to selected objects

Object context menu

The appearance, behavior and position of inserted object can be changed via the context menu that appears by right-click:

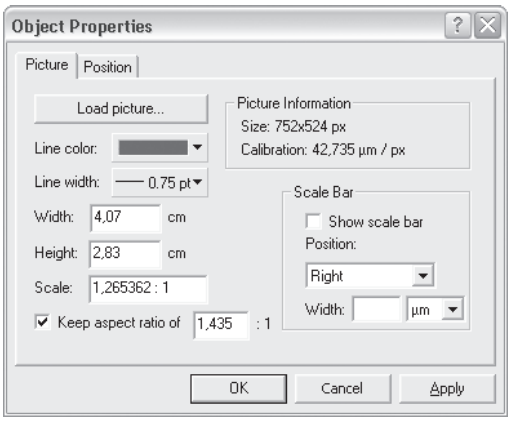


- The **Align or Distribute** command enables moving objects within the page or align them to another object.
- The **Resize object** command enables to resize two objects to the same size.
- The **Bring to front** command changes the order of overlapping objects and brings the current object to the front.
- The **Send to back** command moves the object to the bottom layer.
- The **Lock** command locks the object disabling any further changes to it until it is unlocked.
- The **Properties** command can change object properties such as: color, background color, border size and color, object size, font (text box), aspect ratio (picture), alignment (text box), and shape (arrow).

** - Please, refer to the Edit>Insert Arrow command for the object properties description.*
*** - Please, refer to the Edit>Insert Text command for the object properties description.*

Object properties

Working with objects



This is an example of the image properties control. The same dialog window appears every time you double-click an object inserted to the report. The **Load Picture** button enables you to load another picture to the picture frame.The **Line color** defines the color of surrounding rectangle. The **Line width** defines the thickness of surrounding rectangle.The **Width** and **Height** controls show the image dimensions. The **Scale** shows the *actual scale:original image dimensions* ratio (the image must be calibrated to use this feature, e.g. scale 1:1 means, that the image will be printed in the original size).If you check the **Show scale bar** box, the scale will be displayed. The position and width of the scale bar can be defined. The **Keep aspect ratio of** keeps the original width/height proportions of the image unchanged.

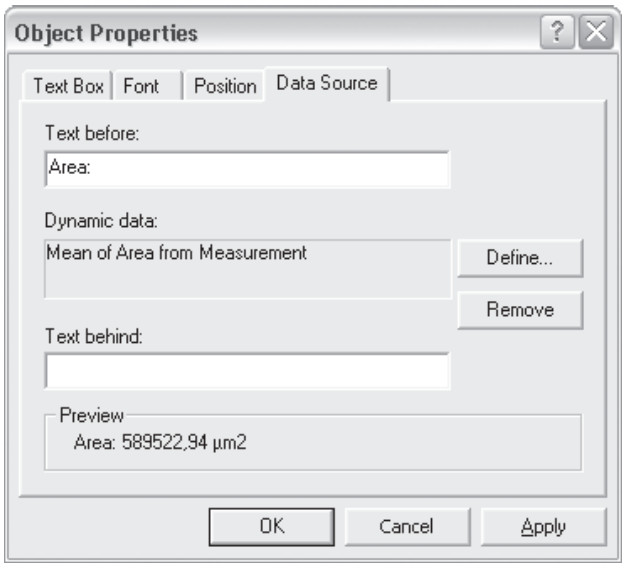
There are other possibilities: You can align two objects to the same horizontal or vertical level. Select more objects (e.g. by holding down the CTRL key and clicking with the left mouse button). Then, pressing the „Align or Distribute“>“Align Top“ command from the context menu will align the objects to the same upper-horizontal level. Other buttons will adjust objects in horizontal, vertical or both directions. If you have checked the „Align to Edges“ button (the last button), then the images will be aligned to the edges or the center of the page.The same can be done using the **Resize Objects**. Select more objects and select the „master“ object to which size you want to resize the other objects.

Creating report templates

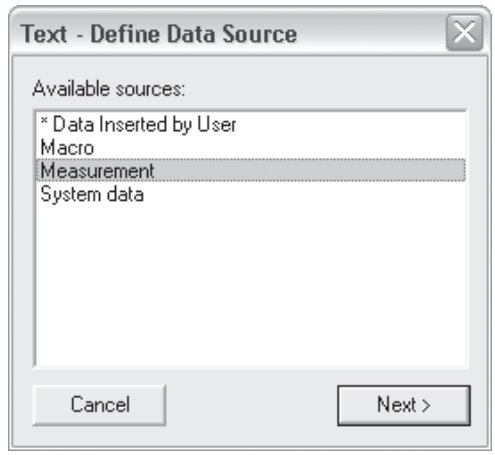
Working with templates

A more advanced task is to create reports, which contain some measured data. You can measure the data in the interactive measurement mode. Then you can make a report reflecting e.g. the mean length value of some objects, the area distribution histogram, or a list of all measured data in a table. For such a task a report template must be created. This template contains the definitions how the report should be dynamically created. The report template, once stored to the hard disk, can be run at any time on any data set resulting in creation of reports with up-to-date measurement figures.

Invoke the **New Template** command from the **Report** menu. An empty report window will appear. Then insert a text box or a table. Invoke the object context menu and invoke the **Data Source** command. The following window will appear:

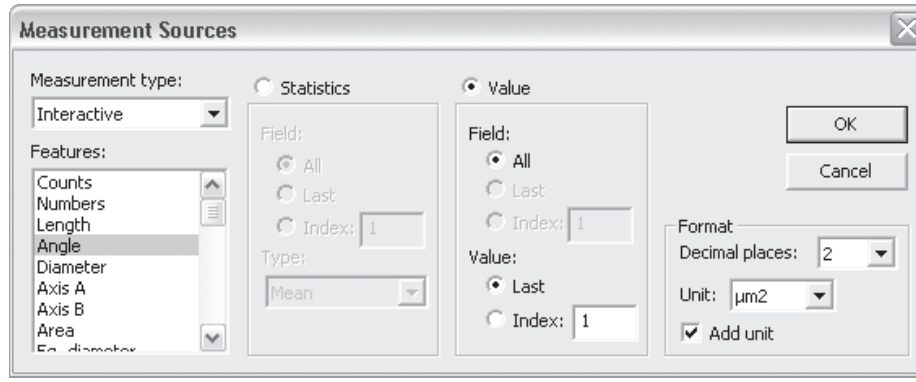


Click the **Define** button. The following window will appear:



- The **Data inserted by user** option means that the system will ask you to type text during the report creation. This text will be added to the report. There will be a prompt for text at the time of report generation.
- The **System data** option enables the insertion of some general data from the system (Date, Logged user or page number).
The **Macro** option enables the insertion of espressions, values, or results of the macro that is running simultaneously.- The **Measurement** option enables the insertion of interactive measurement results as already discussed.
- The **Database** option (displayed only when creating the report from a database) enables the insertion of a link to a database description. This option is used mainly with database exports and it is recommended to use it with the database export wizard as discussed in the following text concerning the database exports.

Choose the **Measurement** option and press the **Next** button. In the next window, you will have the option to define the data source:

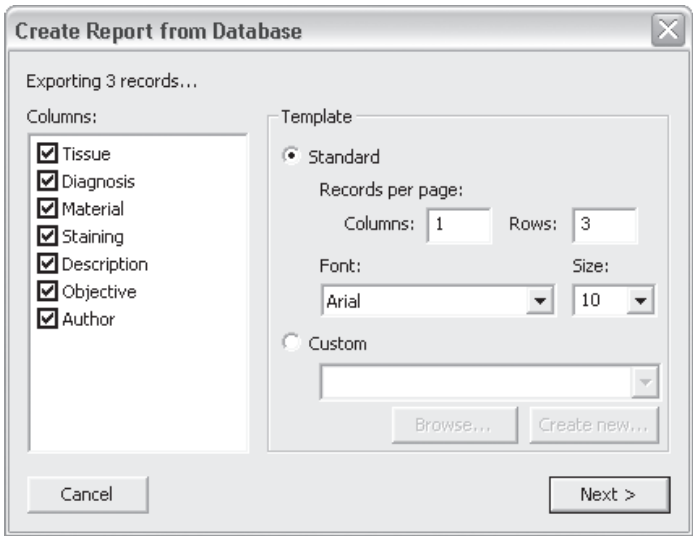


In the left column, choose the interactive measurement feature e.g. Length or Equivalent Diameter. This feature will appear in the report. Then you can choose **Statistics** from a set of values or a particular **Value**. The **Field** defines the range of data used for the statistics calculation. The **Type** of statistics offers: mean value, standard deviation from dataset, median, minimum value and maximum value.

Report from database

Exporting from database

A straightforward reporting task is exporting the database pictures together with the corresponding text info.The easiest way is switching to a thumbnail view by activating the **Organizer layout**. Then click the **Report** button. All selected images are exported. To export the whole database, select all images displayed. The following window will appear:



In the **Columns** section, you can select the text info fields, which will be included in the report. If you are exporting the images from database, you can select database fields. If you are exporting images from a directory, this enables you to select the image text description fields (applies only to images with text info: JPEG2000, LIM and TIFF).Template section defines the report template. If you select the Standard template, images will be organized in rows and columns.It is possible to choose the number of columns (recommended value max. 3) and number of rows (recommended value max. 6).

If you select the Custom report template, then a user report template (*.rtt) can be opened from the disc and used for the report creation.If there is an existing report template, click on the Browse button, otherwise click on the Create new button.The wizard will appear and you will be able to select the number of columns and rows on the page and resulting paper size and orientation.Then a page containing a grid will appear. In the upper left corner of the gird, there will be the picture box and text boxes.You can resize the boxes to wished size. You can also change the boxes mapping. This is an advanced feature, which will be discussed later. What is important to note, is that while editing the report template, you should edit only the objects in the left upper cell of the grid.Content of the other table cells will be derived from the left upper cell during the report creation.

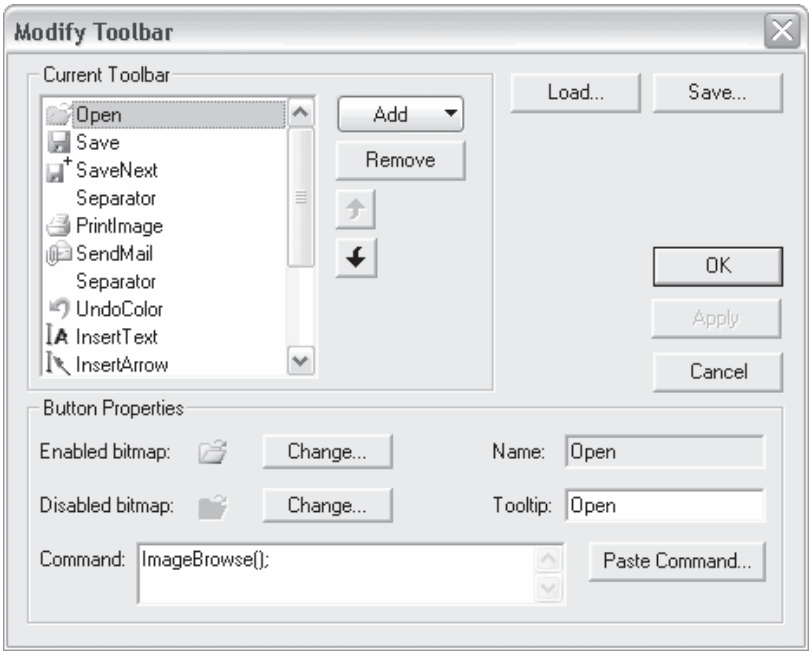
The advantage of custom template is that one can create for example reports with user definable header, which is located at the top of the page placed above the grid. Another advantage of custom templates is the ability to insert automatic page numbers.

Customization

Modifying Main Toolbar

Adding buttons to toolbar

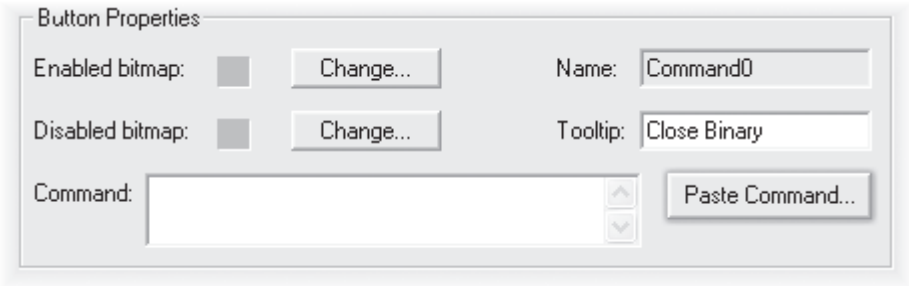
NIS-Elements enables the adjustment of the **user toolbar**. You can define your own buttons to perform various actions like invoking commands from menus, running application functions or executing a macro. To adjust existing toolbar, right double-click any button on the vertical user toolbar on the bottom-left side of the screen. Or, go for the menu command „View > Customize Toolbar > Setup“. The following dialog box will appear:



Lets say that we very often use the **Close Binary** function. It is useful to add the button with the shortcut to this function to the **user toolbar**. First invoke the **Modify Toolbar** window. Press the **Add** button, and choose **Command** from the context menu:

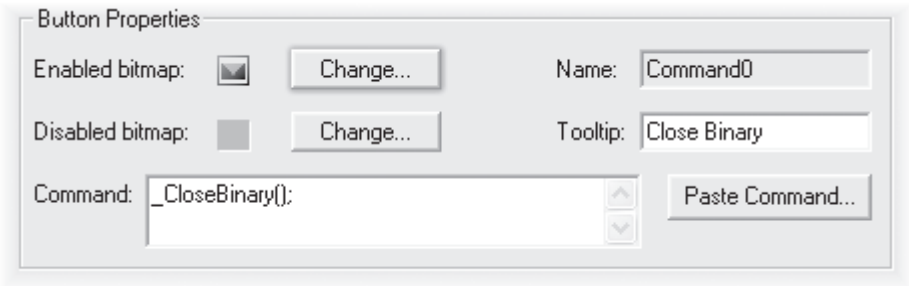
The new command (Command0) is added to the Current toolbar list. Now, attach the NIS-Elements command to it. In the Button properties area click the **Paste Command** button. Choose a command from the list (_CloseBinary() in our case). It appears in the Command

edit-box. It is possible to create a sequence of commands, by using the **Paste Command** button repeatedly.



Changing icon

If you are not satisfied with the default icon given, you can change it by pressing the **Change** button (shown on the picture bellow). A dialog window for selecting the icon appears. We choose the icon that is used in Binary editor for the **Close** function. You can select NIS-Elements icons by default or load some from other files containing icons (typically *.ico or *.dll files). You can define another icon to be used for your command in a disabled state.



Inserting tooltip

It is handy to define a tooltip (a text that appears when placing a mouse cursor over the icon) for your command. Simply write the text into the **Tooltip** box. You can change the position of the command in toolbar by using the arrows located under the **Add** and **Remove** buttons.

You can save your toolbar for further use by clicking the **Save** button. A dialog box appears. You just define the name and press **OK**. You can delete an already saved toolbar by pressing the **Remove** button. To load your saved toolbar use the **Load** button. A dialog window with all saved toolbars plus one default appears. Select the toolbar you want and press **OK**.

Adjusting buttons appearance

Alternative button appearance

You can select between two modes of the buttons display. One is so called **flat look** (that is set as default). Buttons are displayed as flat and they raise only if you move the mouse over. The other is standard, known from earlier versions of NIS-Elements, with standard buttons. To change it invoke the **Options** dialog, pane **Appereance**, by double clicking the NIS-Elements title bar. There, uncheck the **Flat look** box in the Appereance/Handling section.

Command Line Options

When starting NIS-Elements from the command line (or when editing the desktop shortcut) you may take advantage of using the following parameters:

Syntax

Syntax:

executable [switches] [filenames]

E.g. **„C:\Program Files\NIS-Elements\NIS_AR.EXE“ -f „C:\Images\starting_image.jp2“** runs NIS-Elements and opens the *starting_image.jp2* at the beginning.By default only one running instance is allowed. When another instance is already running, the command acts on that running instance (if not changed by the -q switch).

Switches

Switches:

-? [no parameters]
Displays a help screen with the following content.

-c „Command“
The application runs an interpreter Command.

-f „Filename“
The application tries to open an image pointed to by Filename.

-g „Grabber name“
Grabber of the specified Grabber name will be used. No dialog box is shown. As the name of the grabber, you can use the name which appears in the startup driver selection dialog, or the name of the driver class.*See registry entries ...Laboratory Imaging\Platform\Platform.INI\CLxGenericProviderGrabber.*

-gn [no parameters]
No Grabber used.

-h „HW Unit Name“
A specified HW Unit will be used. When using this option you must also specify the grabber name. Specifying last as a HW Unit will cause the application to use the one selected last time.

-i „Config Name“
Application will use the specified Config Name to load from and save to its configuration.

-l „Language“
Application will run in a specified language. The following languages are recognized (ordered alphabetically): Czech (or CZE), English (or ENG), French (or FRA), German (or GER), Italian (or ITA) and Polish (or POL).

-m „Macro File“
Application will execute a Macro File.

-p [Command]
The application window will be placed according to the parameters:

When [Command] is:

- **left** the window is placed on the left monitor.
- **right** the window is placed on the right monitor.
- **top** the window is placed on the top monitor.
- **bottom** the window is placed on the bottom monitor.
- **monitor N** the window is placed on the Nth monitor.
- **rectangle (x0,y0,x1,y1)** the window is placed in the specified rectangle (in workspace coordinates). The coordinates must be in parenthesis without any spaces.

-q [no parameters]
A new instance is created.

Additional Modules

Database Module

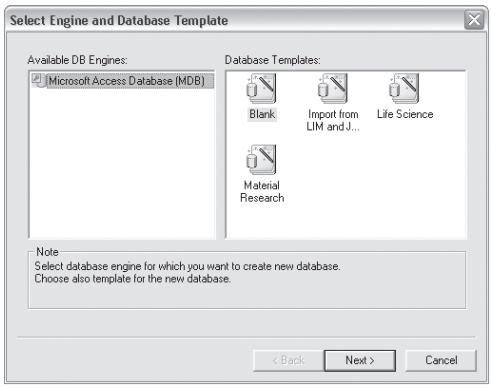
Database Overview

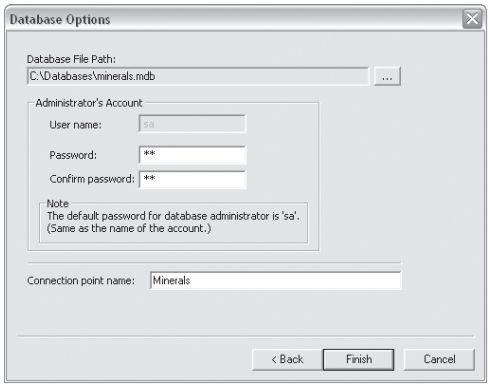
NIS-Elements offers a database tool to efficiently manage images along with additional information. After you install the Database module, the **Database** menu appears in the main toolbar.

A database is an organized body of related information. It consists of one or more database tables filled with data. Before you can start filling the tables, you need to create a new empty database. This can be done by selecting the **Create database** command from the Database menu.

Creating database

Select the Microsoft database engine in the left list and the Blank template from the list of templates. Click on the **Next** button.

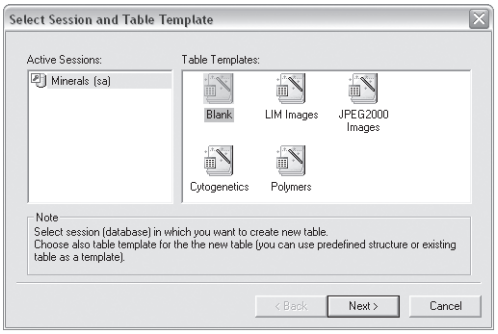




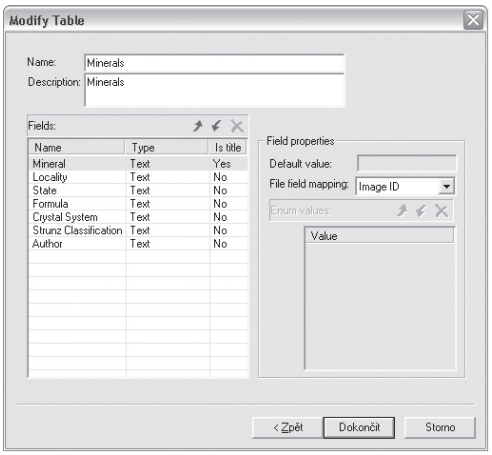
Specify where the database file should be stored on a disk - define the **Database File Path** („C:\Databases\minerals.mdb“ for example) and the database file name. You, as a database creator, are automatically considered as an administrator (the user name and password are „sa“ by default). The administrator is a user with all possible rights to the database. Next, define a descriptive name of a connection point, e.g Test for the test database. Click **Finish** and an empty database is created and connected through the connection point.

Creating tables

A database can contain one or more tables in your database to store your data to. Select the **Create table** command from the **Database** menu.



Select the session from the left list. In our case, there should be only one: „Test(sa)“. It means: the user „sa“ is connected to the database through the „Test“ connection point. It is posssible to have one user connected to more databases or more users connected to the same database on one system. On the right side, choose the **Blank** template and click the **Next** button.

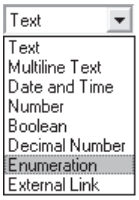


Enter the **Name** of your new Table (e.g. Minerals). The **Description** editbox isn‘t necessary to fill, but it might prove helpfull to put down a short comment.

Define the table fields. First, click to the **Name** field and fill it in (e.g. Author). Press **Enter** and define the data type of this field. This is done by selecting one option from the combo box, which opens after clicking into the **Type** field. Select the **Text** type, because names are usually short enough to fit in this data type. The **Is title** field determines, which database entry is used as a description of images while viewing the table using a thumbnails view of the Organizer Layout. Define all fields you want to include in the database table and click **Finish**. The new table is created.

Special field types

Let‘s assume that pictures come from a closed group of people. In this case it is usefull to set the field type of the „Author“ field to **Enumeration**. The **Enumeration** value edit box on the right becomes active and you can enter all names of possible authors. When adding a new image to the database later, you will just select the author`s name from the combo box instead of having to type it.



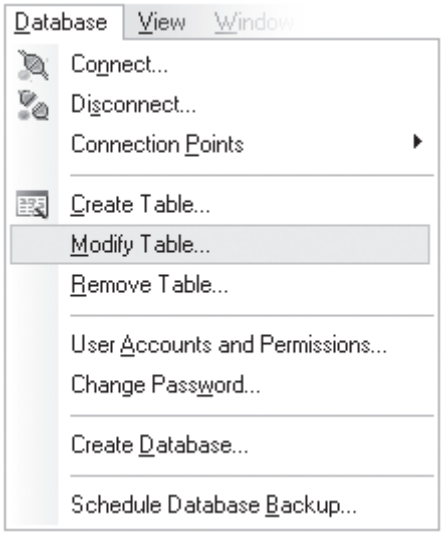
Another special field type enables to insert arbitrary external links into database tables. Select the **External Link** option as the field type. Later, you can insert a command, link to a website etc. to these fields.

File field mapping

It may be very handy to insert some image properties to the database. Therefore the **File field mapping** can be used to map file properties (such as filename, file date, calibration, author etc.) included in the image file to the table fields. Select the information type from the combo box. If the **<default value>** is selected, fill in the **Default value** text box and that text will be used for every database entry added to the table.

Managing tables

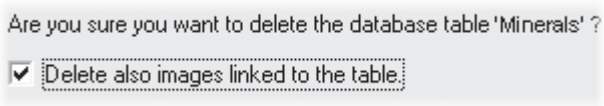
After you have created one or more tables, you can manage them easily using the **Remove Table** and **Modify Table** commands from the Database menu. Each command opens a dialog window.



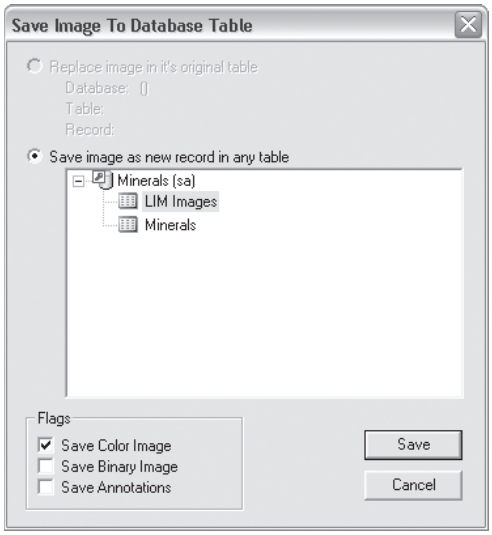
The **Modify Table** command asks you to select a table to modify and opens the table properties window.

The **Remove Table** command removes a table from a database. However, the database images may optionally not be deleted. A directory to store images of each table is created during the table creation. The directory has a name that matches the name of the table and is placed in the directory where the database file is stored. Copies of the source files from your disk are being created in there for database purposes.

If you check the „Delete also images linked to the table“ check box during the table removal, all these copies are deleted from your disk. Otherwise, the copies remain untouched.



Adding images to database



You have successfully created a database with at least one table. Use it to store pictures with descriptive information. Every time you choose the **File** menu **> Save to Database** command, you will be prompted to pick a table to write in. The **Save** button invokes a window to fill the database entry information and saves the current image into that table. View the database records in the Organizer View (the F10 hotkey).

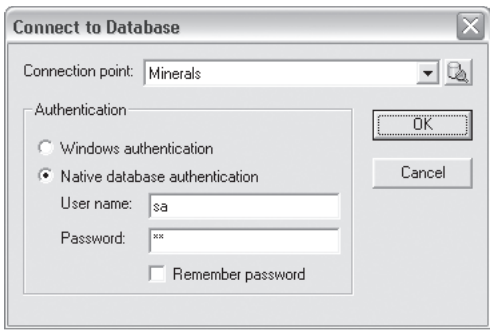
Connection points

Connection points are intended to ease work with databases. A connection point has a similar functionality to a file shortcut or a link. It can be named arbitrarily and you can access the database through it. It is allowed to create and use one connection point per database here.

Let`s suppose you have a database created. Launch the **Create Connection Point** command. Write the connection point name and select the path to the database file in the wizard that opens (once you have created the connection point, you can manage user accounts and permissions of the related database by selecting the **User Accounts and Permissions** command from the Database menu). You can also create more or delete some connection points in the CP manager (Database menu > Connection Points submenu > Manage Connection Points command).

Connecting to database

Use the **Connect** command to connect to a database. Select the connection point (representing the database) from the combo box. In case this combo box is empty, press the icon on the right side to create a new connection point.



Authentication

To acces the database, a user authentication is needed. If the **Windows authentication** option is chosen, the username and password of your windows account are used when logging in. The **Native database autheticati-on** requires entering the username and password which is valid for the database you want to connect to. Click **OK** to connect.

Active connections

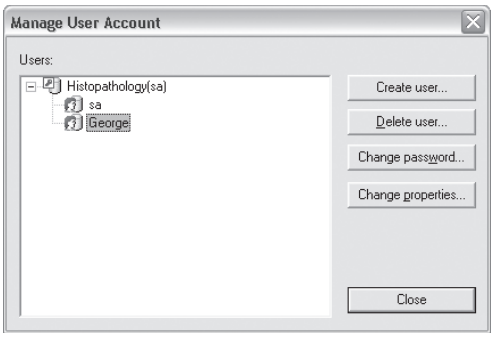
You can view the list of active connections in the Organizer layout (F10 key shortcut). The **Disconnect** menu command displays the dialog box where you can select a connection point to be disconnected. Disconnecting the point closes the way to save images to the corresponding database for the user. Still, you can access the other databases through other connection points.

Every user who possess rights to the database administrator account (with the username „sa“ by default) can manage other user accounts using the **User accounts and permisions** menu command.

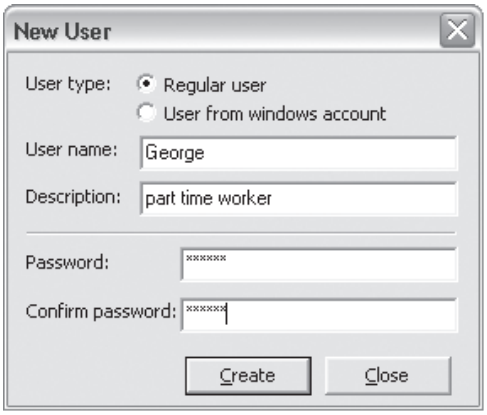
User accounts and permisions

The **Create user** button displays a dialog box (see below). The **Delete user** button displays a confirmation dialog box - when **OK** is pressed, the user is deleted.

The **Change password** button enables to set a new password for the selected user. The dialog box with two editboxes (a new Password and its Confirmation) appears. The **Change properties** button opens an advanced dialog box where you can set various user rights.



Create new user account



The user creation allows you to choose between the **Regular user** (this option enables to create an arbitrarily named user) and the **User from Windows account** (it is possible to choose from users, who have a windows account on the current computer).

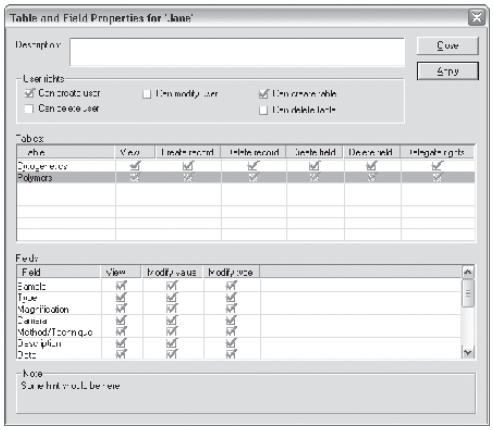
Write a new user name to the **User name** box or select existing user from the list of windows accounts. You can add any comments to the **Description** box. Enter a **Password** twice (**Confirm Password**) and **Create** the new user.

Note

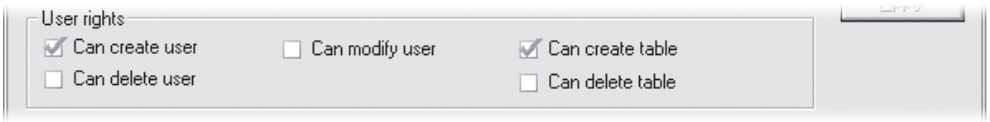
Multiple connections to databases using different user accounts are permitted. If some intended operations are unavailable, check whether you are logged under the correct user account with adequate rights.

User permissions

Having sufficient privileges, you may change the other user`s accounts properties by pressing the **Change properties** button in the **User Accounts and Permissions** window. The dialog box, where it is possible to set user rights for various actions performed on a database, opens. To set or ban the rights, click into the check boxes. There are three main sections in the dialog window: the **User rights**, **Tables** and **Fields.**



The User rights section

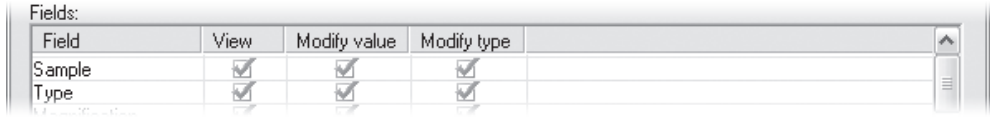


This section is used to define, what actions are allowed for the selected user in a relation to other users and all databases. Decide, whether the user can delete, modify, create user accounts or delete and create tables. The „**sa**“ (database administrator) user`s properties cannot be modified nor the account can be deleted by other users.

The Tables section

Displays a list of existing tables. It is possible to set rights to view, create and delete whole records, as well as rights to create and delete single fields of a table. When the **Delegate rights** check box is checked, the user is allowed to modify permissions of other users for that table.

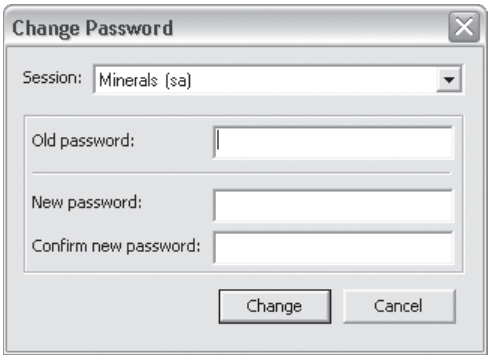
The Fields section



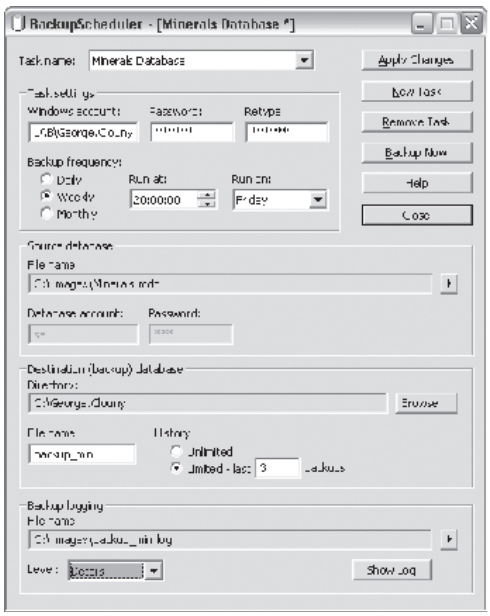
More detailed settings for each table are listed here, if the table was selected in the Tables section. You can only see the fields that your privileges allows you to view. You can set, if the user will be able to view or modify every single field.

Changing password

You can change a password to a database via the **Change password** command from the Database menu. Enter your old password, and a new one twice to prevent a typewriting mistake. The **Change** button finishes the operation. A user with a privilege to „Modify user" can change the other user`s passwords via the **User Rights and Permissions** menu command.



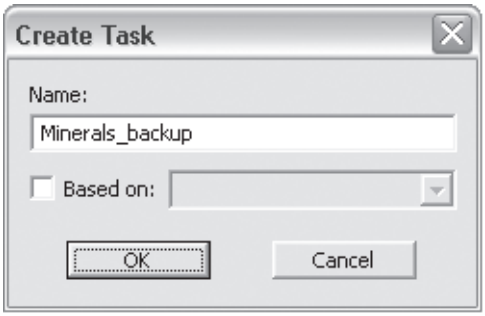
Database backup

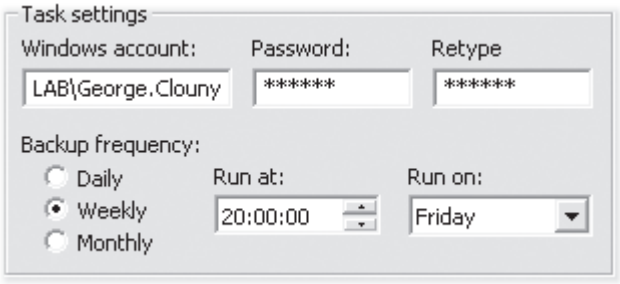


The possibility to backup the database is essential and strongly recommended for any serious work. The **Database Backup Scheduler** enables the system to backup the database automatically once in a precisely specified time interval, so you do not have to be afraid to loose any data. The Database Scheduler uses the standard Windows „Scheduled Tasks" tool (Start > Control Panel > Scheduled Tasks). Once you schedule a backup, there is only one condition to perform it succesfully: The computer must be **ON** at the scheduled time.

Backup settings

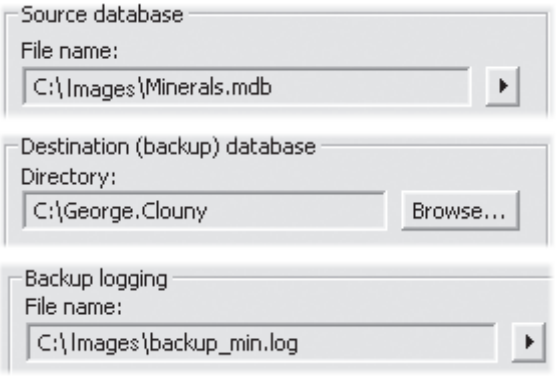
To set a new periodic backup, click the **New Task** button. In the dialog window that appears, specify the task`s name and click **OK.** If you already have some task that suits your needs, it is possible to copy its properties by checking the **Based on** check box and choosing it s name from the list.





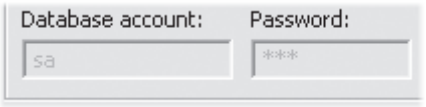
Since the task is based on the Windows component, it is necessary to run it under a Windows user account. Either a local or a domain account is allowable (the domain name „LAB“ is used in our example). After filling in the password, decide, how often the backup shall be performed. Daily, weekly and monthly intervals with a precision of seconds are available.

Furthermore, the location of three following files on a disk is needed. Use the **Browse** buttons on the right side of the textboxes to specify the paths to the **Source database file** (the file to be backed up), the **Destination (backup) database** (a directory to which the backup file will be written) and the **Backup log file** where the information about the backup process is stored. The specification of all three destinations is mandatory. Without it, the backup cannot start.



Detailed backup settings

In the Source database settings, there are the **Database account** and **Password** text boxes. They are not editable and are for information only.

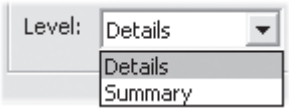


Backup history

When performing a backup periodically, the already existing (the latest) backup file`s content is moved to a new history file and a suffix with the iteration number is attached to its name. The new backup file is created. This way, an unlimited number of the history backup files is being created on a disk. When you limit the number of history files (e.g. to prevent the disk capacity overflow), each time the scheduler replaces the latest file with the new one, it also renames all files accordingly. As a result, the file names and their number on a disk remain the same (although their content changes).

Logging backup process

The amount of information to be written to the backup log file is determined by the **Level** choice. The **Details** option records changes of all files of a database compared to the previous backup. If you choose to record the **Summary** only, the possible critical errors and backup process results are included in the log file.



Extended Depth of Focus Module

EDF overview

The EDF module allows you to combine existing Z-stack images into one focused image picking the focused regions from each frame and merging them together. The „Allign“ algorithm ensures correct fitting of pixels even for images acquired via macro optics, where objects are slightly shifted or scaled during the z-stack acquisition.

When you start NIS-Elements with the EDF module installed, four new items appear in the Applications menu:

Open (or acquire) an ND2 document that includes the Z dimension.

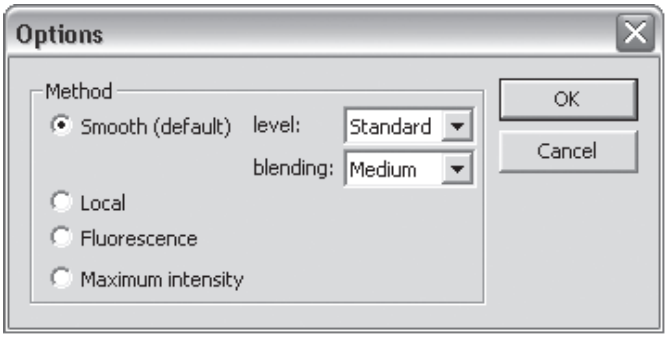
Align Sequence

Small shifts of the images can occur as a result of hardware inaccuracies during the Z series acquisition. To get reliable results out of the EDF module, the sequence that is to be processed should be aligned first. Use the **Align Sequence** command to automatically correct possible shifts.

EDF options

Select the method

Once the sequence is aligned, invoke the **Options** command to select the preferred method of creating the focused image. The following dialog window appears:



Smooth (default)

The focused image is created step by step. In each step, this method computes the focus criterion for each pixel and compares them. It has two parameters which you can change according to the needs of a sequence.

- **Level** - Affects the smoothness of the focus criteria in the image. Higher settings mean that the focused areas in the image will affect larger neighborhood. The default setting is Standard.
- **Blending** - When the focus criteria in the source frames are similar, the pixels from those frames can be combined and the transitions between the focused regions in the result image will be smooth. The higher blending you select, the more differing pixels will be combined.

Local

This method computes the focus criteria in another way than the Smooth method. It finds the focused regions of the source frames and copies them to the result image. An additional processing of the regions removes the badly detected ones. However, borders (transitions) between some regions might be clearly visible at the end.

Fluorescence

Similar to the Local method, but it uses different focus criteria optimized for fluorescence images.

Maximum intensity

The focus criterion is the intensity of each pixel. The pixels with the highest intensities of all frames are copied to the result image.

Focused Image

Creation of the focused image

When the method is selected and the sequence is aligned, the only thing to do is to select the **Create Focused Image** command. The focused image will be created and appended to the ND2 document (*when the ND2 file is saved to disk the focused image is included.*)

Displaying result

Viewing of the result

After the focused image is created, all buttons of the EDF tool bar (located inside the document window) become enabled. There are three ways to observe the resulting focused image, as:

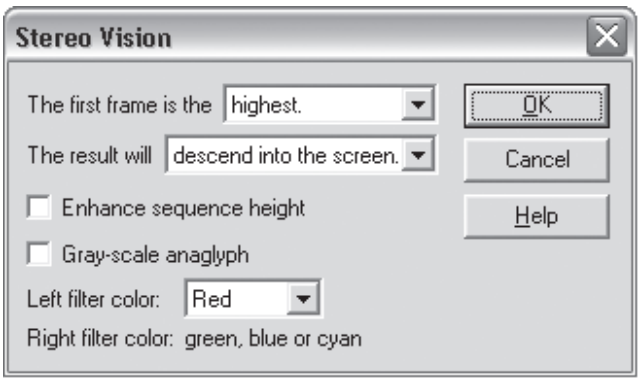
- A common Color/Gray image
- An anaglyph - to be viewed using special 3D glasses.
- A 3D model, using so called Surface View.

This button displays the focused result image.

Anaglyph

Creating anaglyph

Using this button, you can create three-dimensional stereo images (anaglyphs). These images shall be observed with special glasses having red and green or blue filters. This provides a 3D impression. Click the nearby arrow button and select the **Anaglyph options** command.
The following dialog box appears.



The first frame is the

Defines the direction of the z-axis sequence. Can be *highest* or *lowest*

The result will

Defines the stereovision impression, which you will experience using special red-green glasses. Can *descend into the screen* or *rise from the screen*.

Enhance sequence height

Enhances the depth feeling.

Gray-scale anaglyph

Creates a gray-scale anaglyph. If not checked, the anaglyph is color.

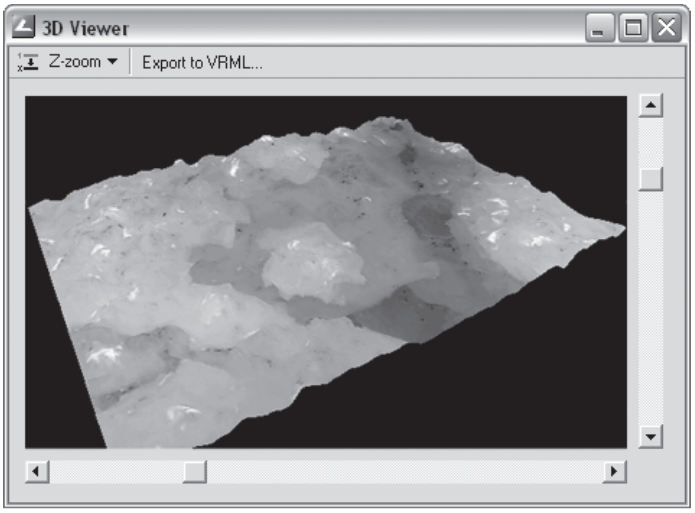
Left filter color

You can select the color of your glasses. This option is crucial to get the right 3D impression.

Surface view

Exploring the 3D model

This button runs the built in 3D viewer.
The 3D viewer displays the surface of a captured object three-dimensionally.



- For proper view, it is necessary to set whether the first frame in the sequence is the lowest or the highest one (set this by the leftmost icon).
- You can also adjust the sequence height for more credible look via the **Z-zoom** combo box.
- Navigate the view by sliders on sides or by mouse. A mouse wheel serves for zooming.

The 3D model can be exported to VRML (Virtual reality modeling language) format for distributing and viewing outside of NIS-Elements software. This format is platform independent and is used mainly on the internet.

To export the image to VRML, press the **Export to VRML** button. Two files will be saved. One that contains the 3D information (the WRL extension) and the other that is a 2D texture (the JPG extension). Mind, that both files must be distributed (or placed on the web) together!

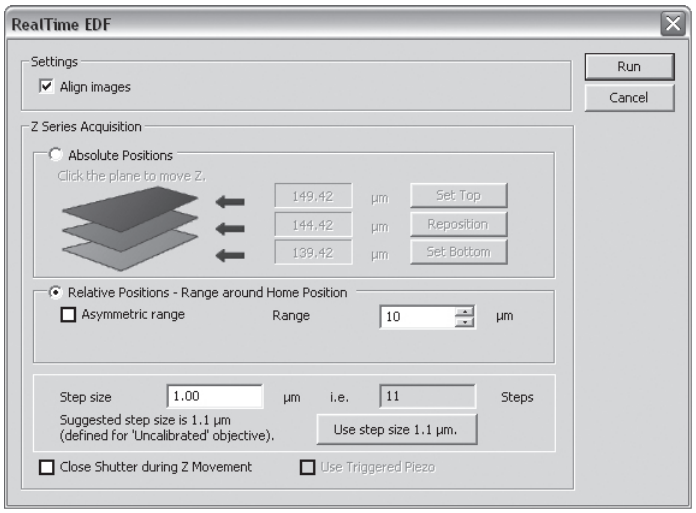
To view files in VRML format some VRML viewer is needed. The viewer is a plugin to your web browser. There are several viewers available for free:

- **Cosmo Player ovrt.nist.gov/cosmo/**
- **Cortona** - **www.parallelgraphics.com**

Real Time EDF

Real Time EDF

The Real Time EDF command is integrating the whole functionality of EDF. It captures a Z-sequence, aligns the images (optionally), and creates the focused image. When the command is invoked, the following dialog appears. Check, whether to align the images or not. Please, see more details about the Z sequence acquisition in the **Z Series Capturing** chapter.



ND Experiments

Introduction

N-dimensional experiments combine up to 4 acquisition types together. Z series, time lapse, fluorescence, and multipoint experiments result in one ND2 file seqence that includes information about the experiment progress.

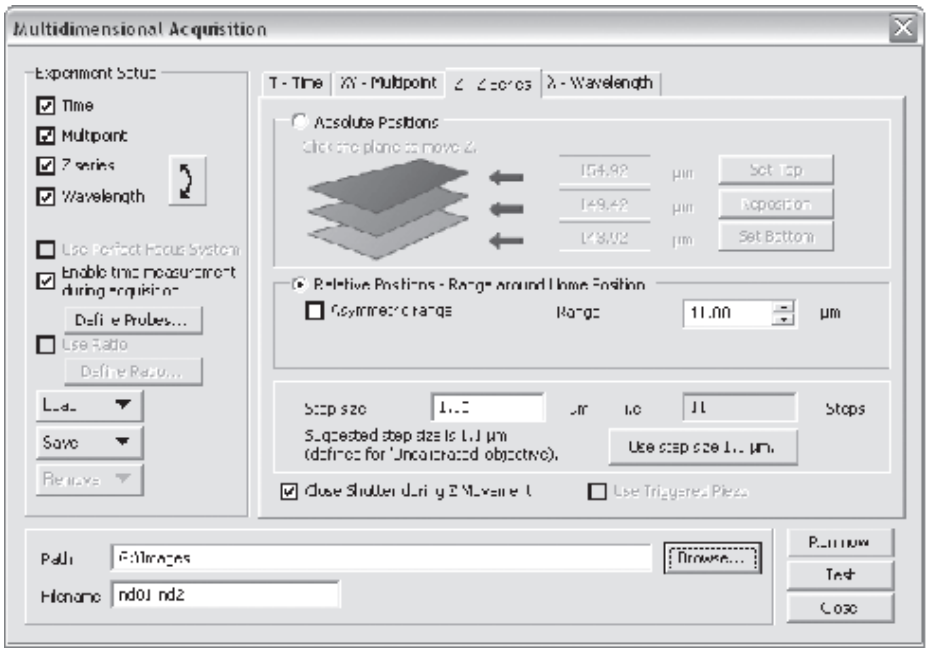
Basic ND

In NIS-Elements Basic Research package, only two dimensions can be acquired within one ND experiment.

There are 2 ways of creating ND documents. Either define **Experiment** and capture it using the camera, microscope and other peripherals or **create it from existing images** by defining the ND document structure. To define an experiment, select the **Define/Run Experiment** command, from the Applications menu.

Acquiring ND documents

Acquiring ND documents



First define the types of „dimensions“ in the Experiment Setup section. You can select from: **Time** - captures images according defined time scenario, **Multipoint** - captures defined regions (x,y coordinates), **Z series** - takes pictures of the specimen at different Z axis heights, **Wavelength** - (also known as Multichannel images), captures the specimen using filters, to get its appearence in various light wavelengths.

You can load previously defined experiment using the Load button or save it using the **Save** button. **Remove** enables you to delete an experiment.

Experiment dimensions

Setting the dimensions properties

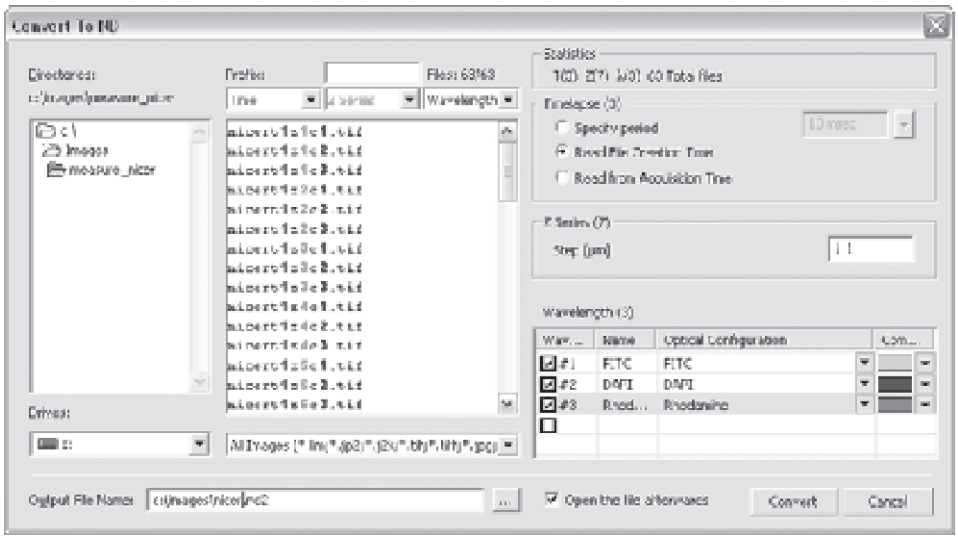
Please, see the detailed description of adjusting the dimension settings in the following chapters:

- **Time Lapse Acquisition**
- **Multipoint Acquisition**
- **Z Series Acquisition**
- **Fluorescence Acquisition**

ND2 document from files

Converting existing images to ND documents

If you have a series of images from a timelapse acquisiton or captured Z stack images, you can convert them to an ND document. Select the **File > ND > Create ND File from File Sequence** command - the following dialog box will appear:



The left part of this window is used for disk navigation. Select the directory, where your images are stored. You can restrict the selection by defining the file prefix and file type. All files matching the given citeria are displayed in the list box. The system automatically recognizes numerical progression in the file names. You see it marked by colors creating virtual columns. You can define which progression corresponds to which dimension. This is done using combo boxes located above the list box. To see if the assigment was correct, look in the Statistic section.

The right part of this window is dedicated to settings of individual dimenstions. **Timelapse** section enables you to set time period between images manually, or use record stored in file (Acq Time) or count the time from file attributes.

The **Z-Series** section offers two parameters. You can set the Starting distance and step between subsequent images.

The **Wavelength** section is used for defining the name, color of channels, and optical configuration that was used for capturing the set of images.

When all parameters are set, specify name and location of the output file and press the Convert button.

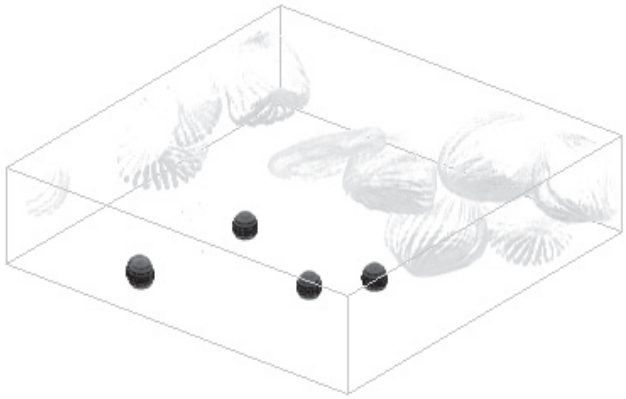
Deconvolution Module

Introduction

Introduction

What is deconvolution for?

Deconvolution is a mathematical algorithm for removing unsharpen parts of image that lays out of the focused plane.



How does it work?

The algorithm uses image information from different Z-slices. The input is a set of Z-slices captured by a fluorescence microscope. The output is another set of slices, but, the image information of each slice that does not belong to its focused plane is suppressed.

The algorithm used is: constrained iterative - maximum likelihood estimation algorithm (the actual name of the algorithm is „Richardson-Lucy“)

Results

The resulting image should theoretically look similarly as if captured by a confocal microscope.

Open image sequence

Getting a sequence of images

Opening an ND2 document including Z series.

Invoke the **Open** command and select the ND2 document to be opened.

Acquiring Z-series

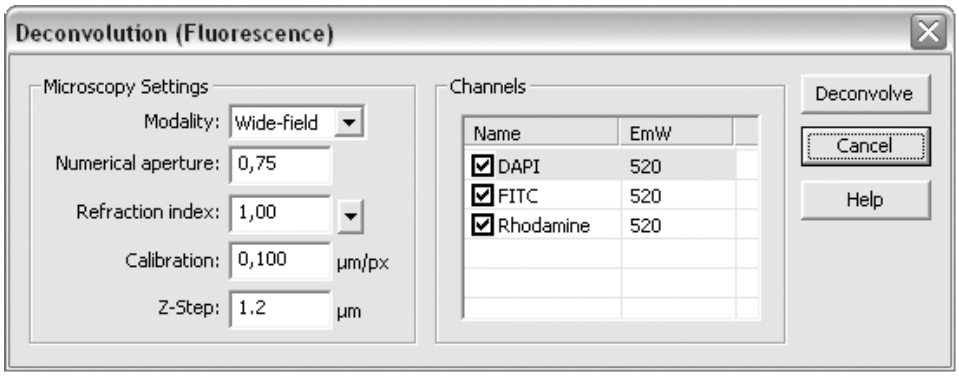
Acquire a sequence of Z slices using the**Capture Z-Series command**. Or, a result of ND experiment that includes Z series can be used.

Selecting EDF method

To deconvolve a sequence of Z Stack images

Run the **Applications>Deconvolution>Deconvolve Z Series** command and set the deconvolution parameters:

To deconvlolve a sequence of RGB images:



- Select your microscopy **Modality**, wide-field or confocal.
- Fill-in the **numerical aperture** (NA) value of the objective used.
- Fill-in the **refraction index** of the immersion medium used. There are some values predefined in the nearby menu (air, water, immersion oil, glycerin).
- Fill-in the calibration field. Mind that the **deconvolution doesn‘t work on uncalibrated sequences**.
- Pick the channels to deconvolve in the **Channels** field.
- Fill-in the emission wavelengths (**EmW**) of the channels. These values don‘t have to be accurate because they affect the result only slightly.
- Fill-in the **Z-Step** distance between the single Z-slices of your sequence.*
- Press the **Deconvolve** button.

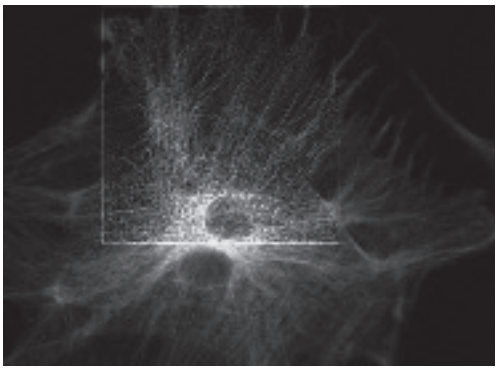
** - It is important to fill the same value that was used during the Z sequence acquisition. The deconvolution will not work properly without a reasonable Z-Step value. When you are about to capture a Z sequence, you should* **set the Z-step value** *to [Suggested step size] or smaller in order to get enough detail for the deconvolution algorithm to work fine.*

The 2D RT Deconvolution Module

Introduction

Deconvolution is an image processing technique that removes inaccurate features in an image that are caused by the optical path. It is mainly used for enhancing blurred images. RT Deconvolution algorithm enables high speed processing, therefore it is very suitable for use on Live signal from camera. Real-time deconvolution can be apllied to Live image or only when capturing. It can be also used for processing of saved images.

There is an example of Real time deconvolution applied to rectangular region of interest:



When you start NIS-Elements with the 2D Real-Time Deconvolution module installed, the RTDeconv section appears in the Applications menu bar with the **Show RT Deconvolution Setup** and **Enhance Current Image...** commands. Click the **Show RT Deconvolution Setup** command to display the following control window.

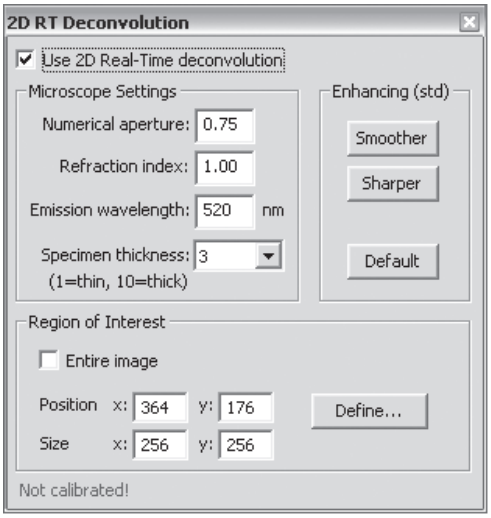
2D RT Deconvolution setup

Use 2D Real-Time deconvolution

This option switches on/off the deconvolution on Live image. When a static document is opened, the **Apply** button appears instead of this option.

Microscope Settings

Microscope Settings are the input parameters for the deconvolution.



Numerical aperture

NA of the objective lens. This value is written on the objective itself.

Refraction index

Refraction index of the lens immersion medium (typically 1.00 for air, 1.33 for water, 1.51 for immersion oil, 1.61 for glycerin).

Emmission wavelength

Emission wavelength of the fluorescent specimen in nm. Use 520nm if unsure.

Specimen thickness

Describes how thick the is the specimen being imaged. The value is typically between 1 and 5. Use 1 for optically flat mono-layers, and 5+ for thicker specimens.

Enhancing

Enhancing group controls the noise amplification vs. image smoothness. The default settingis suitable for low-noise images.

Smoother

Press this button to decrease the noise.

Sharper

Press this button to increase the sharpness of the image.

Default

Return to default (std) setting.

Region of Interest (ROI)

Region of interest specifies the part of the image where the deconvolution will be done. Reducing the area for the deconvolution will increase its speed.

Entire image
Check this option if you want to deconvolve the entire image.

Position (x, y)
Top-left corner of the rectangle representing the ROI.

Size (x, y)
Width and height of the rectangle representing the ROI.

Define...
If you press this button, you can draw and/or adjust the rectangle with mouse directly in the image.

Not calibrated message
It is necessary to have the image calibrated in order for the deconvolution to work. If you see this message, you should calibratethe **optical configuration** you use.

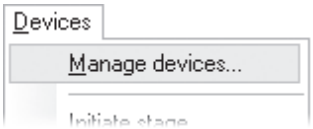
Stage XY Module

Supported devices

The Stage XY Module allows control of XY stages via corresponding controllers. Its functionality includes moving the stage, image stitching, automated scanning and memorizing of the stage positions. The following controllers are supported:

- Nikon 90i + motorized stage
- Prior ProScan controller
- Prior OptiScan controller
- Märzhäuser MCL controller
- Lang ECO-STEP controller
- Lang L-STEP controller

Adding devices to system



In order to begin a work with a device, it is necessary to add it to the **Device Manager** (Devices menu). The Device Manager is designed for adding, removing and controlling hardware accessories and adjusting their settings such as XY/Z motor`s speed, joystick speed, acceleration etc. All the devices are listed together in the Device Manager window.

To be able to add a device, it is necessary to have the appropriate driver installed. This is done during the application installation by picking the hardware to be supported. In the manager, press the Add button and select the device from the list.

Troubleshooting

Any device can occur to be in three different states. Connected, Disconnected or Broken. Each state is interpreted by a different icon in the Device Manager.

- Connected - the device is physically connected, properly configured and communicating with the software.
- Disconnected - the device is not active. Either it is disconnected by user, or the communication settings are incorrect. Check, whether the controller is not turned off and all cables are properly plugged.
- Broken - The device is not responding or reports some errors.

The connecting and disconnecting is done by the Connect and Disconnect buttons of the Device Manager. For a successful communication with the software, all connection parameters must be set properly. Please, see a manual of your device for more details. When disconnecting the device, all configuration settings are stored, unlike when removing the device by the Remove button.

Physical/logical devices

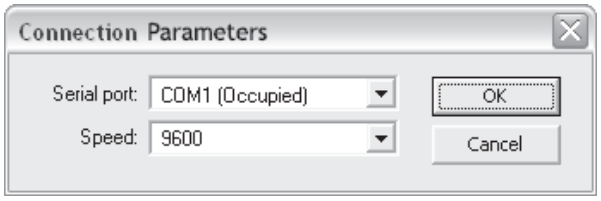
The device manager differentiates physical and logical devices. A physical device is an actual piece of hardware, a logical device is a service that the hardware provides. One physical device can consist of more logical devices. As an example, following schemes are possible:

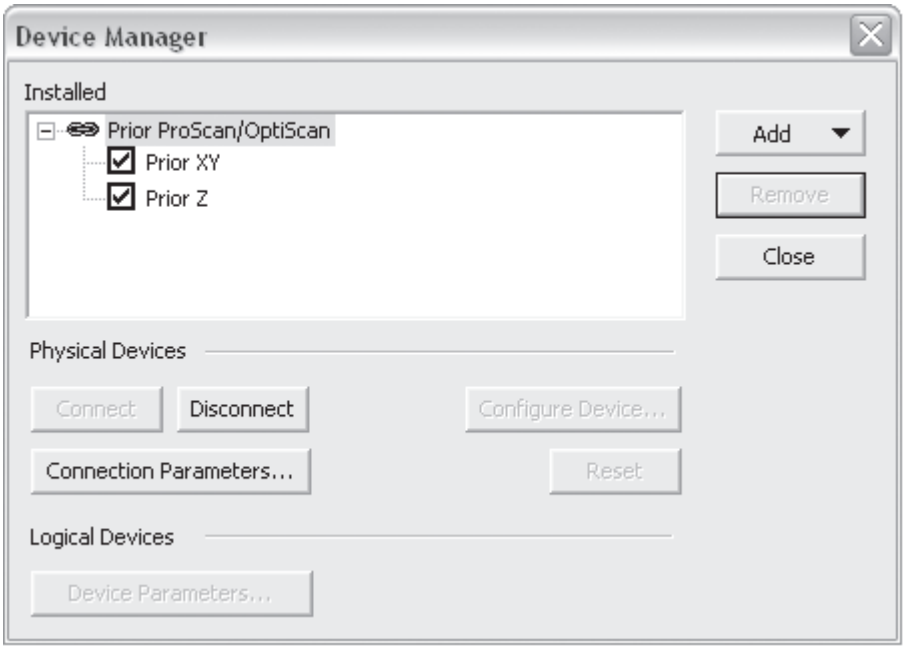
- There can be a motorized Z drive (Z drive logical device) included in the TE2000 microscope and the Prior XY stage (with the stage XY logical device) connected at a time.
- or, there can be only one E600 microscope (without any logical devices) with Prior ProScan motorized XYZ stage attached (stage XY and Z drive included).

Example

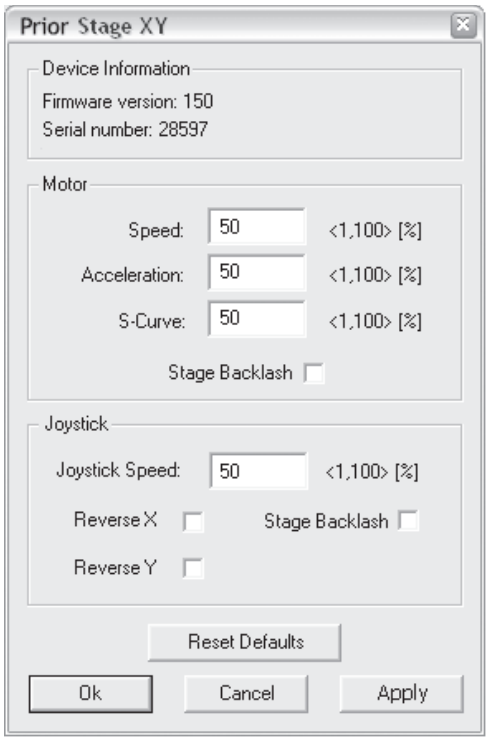
Adding Prior ProScan to the Device Manager

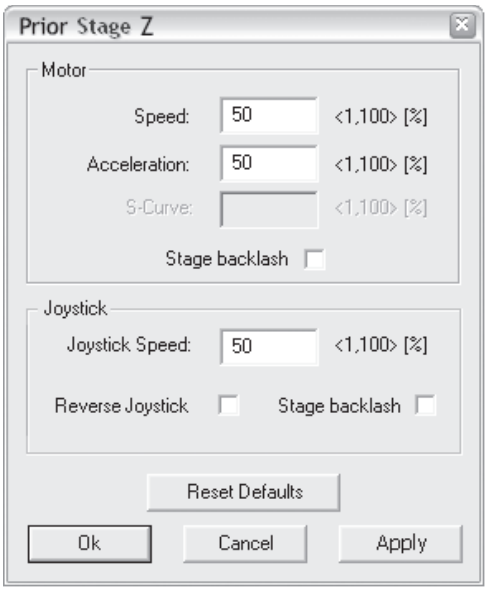
- Reach the **Add** button in the Devices Manager and select Prior ProScan/Optiscan.
- Select the correct serial port to which the controller is connected and press OK. It is possible to change the connection parameters later by the Connection Parameters button. The Prior XY and Prior Z devices appear in the logical devices` list.





The parameters of each logical device can be adjusted using the **Device Parameters** button of the Device Manager, while the device is selected. The following windows serve for modifying the Prior XY and Prior Z devices` settings.





Stage initialization

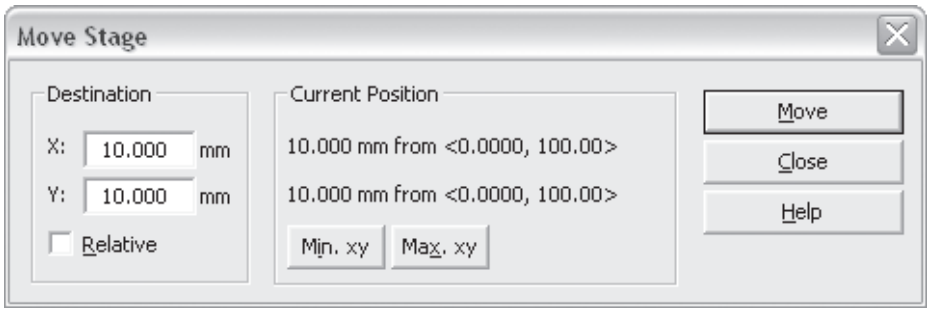
When the stage has been connected for the first time, the initialization is required. The **Initiate Stage** command detects the stage XY movement boundaries. The maximal travel range of the stage is detected.

Caution!

Before the maximal travel range initialization is started, check manually with a joystick, that the stage cannot collide with a microscope objective or a condenser within the whole range!

Moving stage

If you need to control the stage movements with great precision, it is possible to use the **Move Stage** command from the Devices menu.

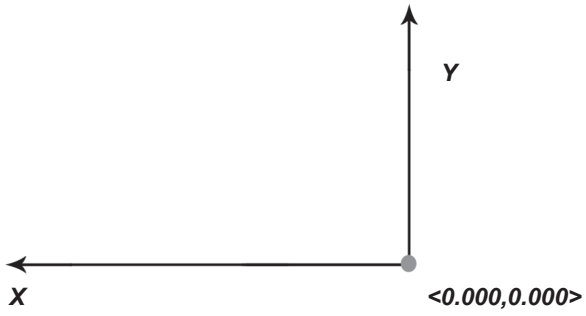


You can move the stage via this dialog window. Fill in the intended destination values and press the Move button. When the Relative check box is checked, the filled coordinates stand for relative position changes.

The Min xy and Max xy buttons move the stage directly to its corner positions.

Coordinate system

The Stage XY module uses the coordinate system, where the origin is in the bottom right corner:

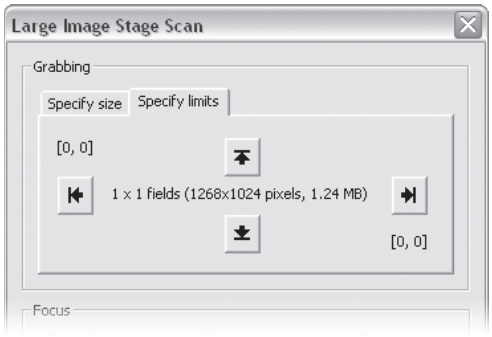


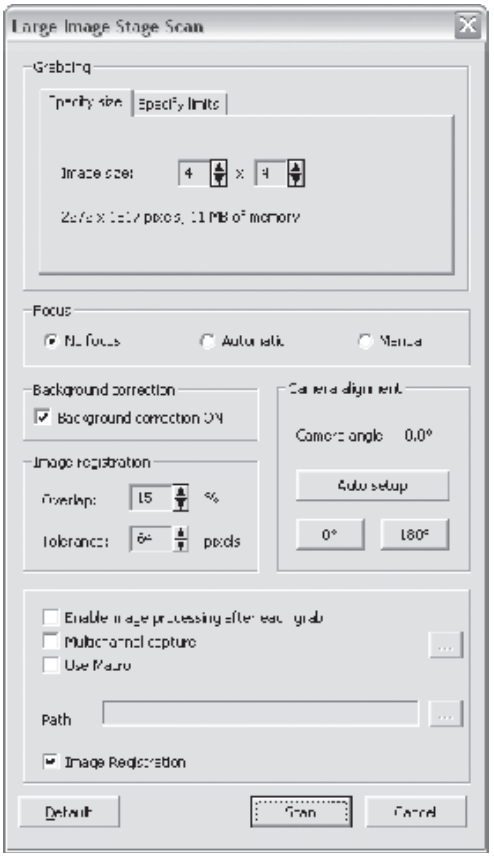
Large image scanning

The Stage XY module enables you to scan a number of images, and stitch them together. The scanning is performed in a similar way to the manual large image grab, except the meander movements are realized via the stage control. To perform this function it is a necessity to set a proper calibration together with the camera angle setting (optionally). This can be done using the stage autocalibration command, accessible from the Quick calibration toolbar (if the stage is initialized). Note that the stage autocalibration and camera angle setting procedures need to have some reasonable scene in the microscope (to be able to recognize the specimen shifts).

The large image scan procedure scans the specimen in the trajectory of meanders, covering the whole area of the rectangle specified by user. The rectangle can be specified either by the current stage position and a number of fields or by specifying the rectangle borders. While setting the scanning area, the user can move the stage using a joystick. While scanning, the system displays a partial result enabling you to interrupt the process by pressing the Cancel button.

Large image scan setup





Before the large image scan can be performed, several settings should be adjusted. Specify the size of the scanning area and stage movement limits in the Grabbing section.

Pressing each button (above), the user specifies that the current stage position corresponds to the appropriate boundary. Or, the large image size can be specified by defining an array of screens to be captured (4x4 in the picture on the right). The estimated picture size is generated.

The method of focusing shall be selected.

- The **No focus** option is usually used for low magnifications.
- The **Automatic** focus is performed before each image frame is scanned. Though, the first image frame prompts the user to focus manually.
- The **Manual** focus is performed by the user before each image frame scan.

If the **Background correction ON** box is checked, the background correction is performed after grabbing. This process is a crucial feature that allows to scan reasonably homogenous images.

The **Image registration** section sets the Overlap of captured images in percents and the Tolerance. The images` Overlap is necessary for combining the images successfully. The Tolerance value defines the range for matching images together.

If you have a nonrectangular camera alignment, the Camera alignment section helps to compensate it while grabbing a set of images.

The **Enable image processing after each grab** check box switches to a special state after each image grab. You can perform various image transformations before the large images are combined together.

If a macro was specified and the **Use macro** option checked, it is run after each image grab.

Selecting the **Image Registration** option activates an algorithm, which tries to find the best position for linking the images up. If you deactivate this option images are linked exactly as they were grabbed.

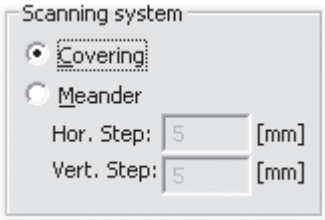
Note

For proper large image scanning, the absolute precise calibration and camera angle adjustment must be done. The precise shading correction is also necessary.

Scanning images

Any defined rectangular or elliptical area can be inspected by the use of the **Scan** command. Or, an array of images can be captured, if you use it together with a macro. Before the scanning starts, set its properties in the **Scanning Setup**.

Scanning setup

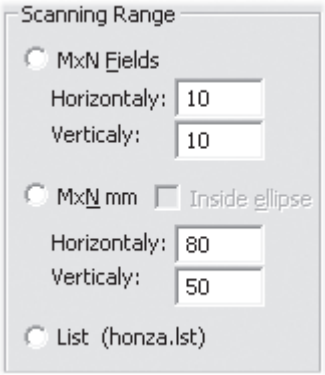


A large dialog window with a number of sections appears. In the first section, choose a scanning system. The **Covering** system scans images throughout the whole defined area. When you select the **Meander** system, a step values can be defined.

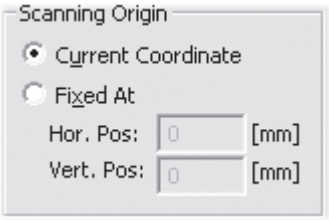
Scanning range

The area to be scanned can be limited in three different ways. Either fill the number of horizontal and vertical fields, or limit the area by defining its real size in milimeters, and, its shape can be switched to ellipse.

The last way is to create a list of positions (Devices menu, Lists submenu, New/Open command).



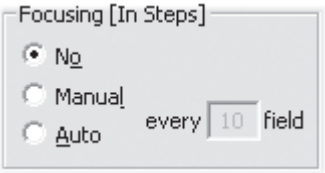
Scanning origin



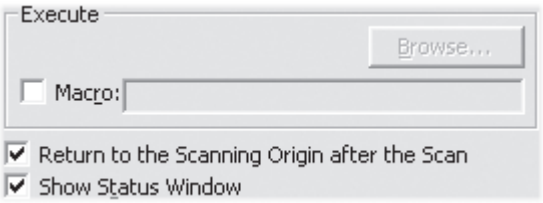
Tell the system, where it should begin with scanning. It can be either a current position of the stage or any point of the maximal range defined by its absolute coordinates.

Focusing

In the **Focusing [In Steps]** section, select **No** to use the current focus throughout the whole scan. Or, it is possible to focus manually or automatically after a defined number of scanned fields (**every N field** edit box).



Other options



If you would like to accomplish more actions than viewing the selected picture areas, you can create a macro to, for example, capture and save images. Check the box and define a path to a macro to be performed after every field is scanned. The **Show Status Window** enables to pause or stop the scanning process via a dialog window. Decide whether the stage should **Return to the Scanning Origin** after all the positions are scanned.

Scanning

The Scan command of the Devices menu starts scanning according to the set parameters. If you have specified any macro in the Scanning Setup, then it is possible to interrupt the scanning cycle with the **Ctrl+Break** keys combination.

Example

An automated scanning is often used for automated tasks, where there is a need to analyze the certain area or there is a need to reproducibly move to certain places. The particle analysis inside the defined rectangular area is a good example. The particle analysis macro must be used as the scanning macro. Another example can be a repeatable inspection of points defined in the user coordinates list. In this case the scaning range is defined by the list. The Scan command is called every certain time periodically.

Lists of points

A List of points allows to move the stage to an arbitrary number of user defined positions. Usually, the list is used to mark specific areas of interest of a microscopic specimen. It can be used for automated scanning too.

Managing lists

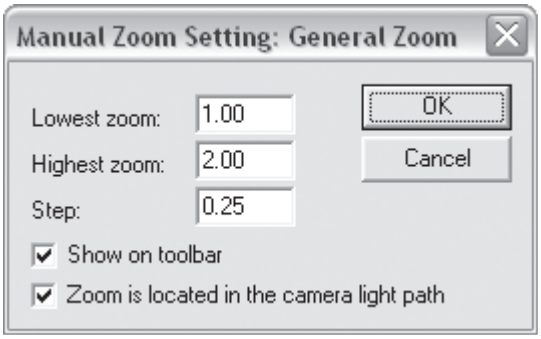
Lists of points can be managed from the **Lists** submenu (Devices menu) by the use of the **New**, **Open** and **Save** commands. A current list`s name is indicated inside the square brackets next to the List command.

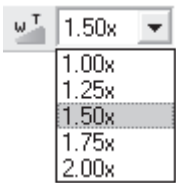
Modifying list

To modify an existing list, choose the **List** command from the Devices menu. A dialog window appears (above). The first five buttons serves for browsing already defined points. Use a joystick or the **Move** command and focus on the intended area. Press the **Add** button. The Delete button removes a current point from the list. The **Reset** button clears the whole list. It is possible to create two or three dimensional lists. If the **Move also Z axis** box is checked, the Z drive positions will be included in the list. The Close button saves the list and closes the window.

Zoom configuration

A usage of a zoom affects the behaviour of many application features. If you have a zoom installed, motorized or manual, it requires to set its parameters correctly. The **Zoom configuration** command defines the maximal and minimal zoom factors. The zoom factor cannot be changed continuously, so there is a necessity to fill in the **Step** value.





The **Show on toolbar** check box displays the following window (on the left) in the main toolbar. A number of possible zoom factors depending on the Step setting is shown in the combo box.

The **Zoom is located in the camera light path** is a crucial option. It tells the system, whether to include the current zoom factor into its calculations or not. The differencies between manual and motorized zoom devices are following.

Manual zoom

Let`s assume that there is a calibrated objective in use. When the zoom factor is increased manually, the image view changes, but the calibration (the real size per one pixel) does not change - even though it should. The software does not have any mechanism to recognize the manual change of the zoom factor. If you capture an image then, it will not be calibrated properly, because the system hasn`t included the zoom factor into its calculations. The conclusion is: **When changing the manual zoom, equal zoom factor must be selected from the zoom combo box**.

Motorized zoom

Working with a motorized zoom is much easier. When the zoom is located in the camera light path and the corresponding check box is selected (above), the zoom can be controlled from the zoom combo box or by the use of a control pad. The process is fully automatic, so the objective calibration is recalculated after each change of the zoom factor.

Stage Z Module

Supported devices

A Z drive enables movements throughout the Z axis direction. It is a logical device, the corresponding physical devices are motorized stages with a Z drive included, standalone Z drives and motorized microscopes with a Z drive included from different manufacturers. The following physical devices are fully supported:

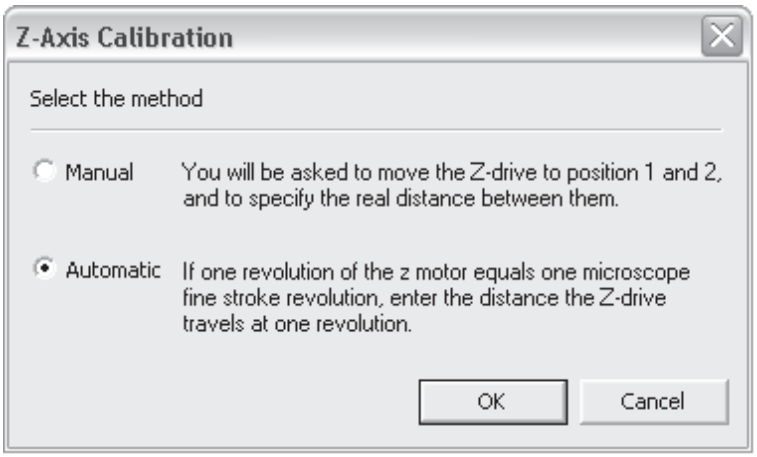
- Nikon 90i microscope Z drive
- Nikon TE2000 microscope Z drive
- Nikon RFA Z drive
- Prior ProScan controller
- Prior OptiScan controller
- Märzhäuser MCL controller
- Lang ECO-STEP controller
- Lang L-STEP controller
- Physik Instrumente Piezo controller

Adding devices to system

In order to begin to work with a Z drive, it must be properly connected and added to the active logical devices list through the Device Manager. Please, follow the instructions in the Stage XY module chapter.

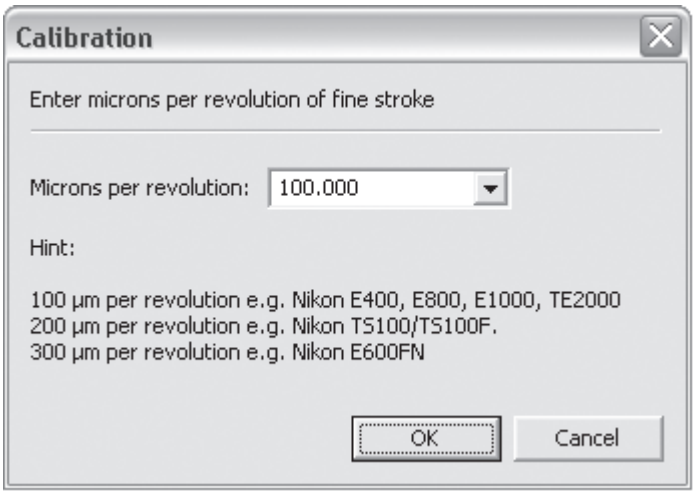
Calibrating

When the Z-drive has been connected for the first time, a calibration may be required. The Z calibration can be also done during the stage initialization or using the **Calibrate Z** command. Launch the command from the Devices menu and select the prefered calibration method. For microscopes, it is convenient to use the Automatic method.


Z-Axis Calibration
Select the method
Manual
You will be asked to move the Z-drive to position 1 and 2, and to specify the real distance between them.
Automatic
If one revolution of the z motor equals one microscope fine stroke revolution, enter the distance the Z-drive travels at one revolution.
OK
Cancel

Automatic calibration

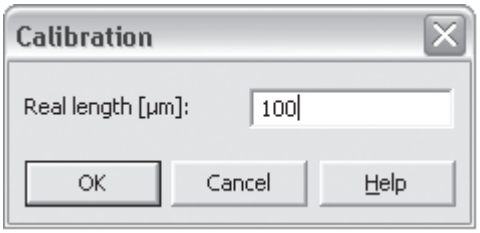
Most of microscopes have a calibrated distance value that corresponds to one revolution of a fine stroke. During the automatic calibration, just select the appropriate distance of your microscope and confirm it by the OK button. It can be 100, 200 or 300 microns.



Manual calibration

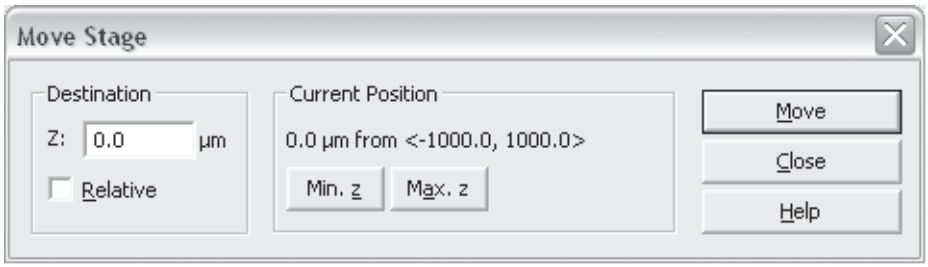
After the first window of the manual calibration process appears, using a joystick, move the stage to the intended position and press the OK button. The second dialog box appears. Using the joystick again, move the stage to the second position and press the OK button. The third dialog box appears, enabling you to type the real distance between these two Z positions.

Type the distance in micrometers. After pressing the OK button, the calibration is finished.



Moving Z-Drive

Various devices can have different means of control connected. In case that there is no other positioning device available, or if you need to control the Z-Drive movements with great precision, use the **Move Z-Drive** command from the Devices menu.

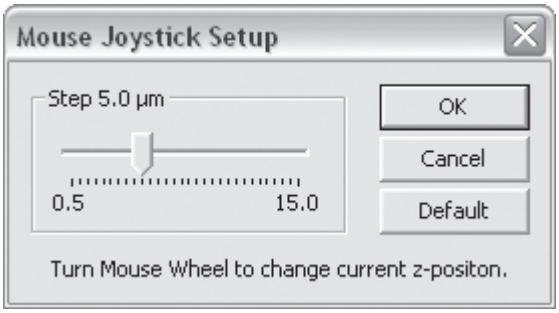


Caution!

There is a risk of damaging the specimen during a careless Z drive movement. If you are not sure what Z position shall be set, keep the Z value to zero and the nearby **Relative** check box checked.

Mouse joystick Z

Another possibility of moving the Z-Drive is to use a mouse wheel. Just enable the **Enable Mouse Joystick Z in Live** function in the Devices menu. The precission of the movement can be adjusted by changing the step via the **Mouse Joystick Setup** (Devices menu).



The step size ranges from 0.1 µm to 50 µm. The Default button sets the Z step of 5.0 µm.

Note

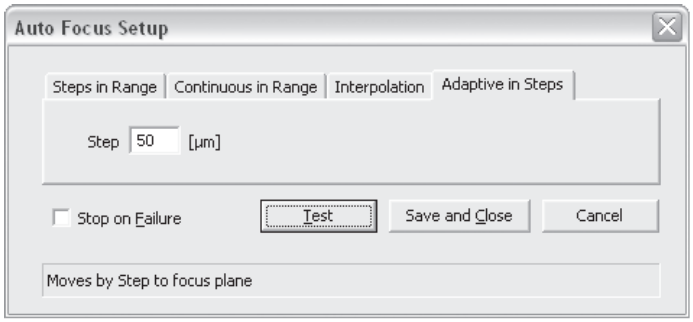
Different objectives require different values of the Z step. For example a 4x objective requires much bigger Z step than a 40x objective.

Auto focusing

The main advantage of a motorized Z-drive is the capability to focus automatically. Four focusing methods can be used. To apply the method that best suits your purposes, launch the **Setup Focus** command from the Devices menu. A dialog window appears. Select one of the methods and adjust its criteria. Press the **Test** button to start the focusing. If the focusing works properly, press the **Save and Close** button. All changes will be stored. The Stop on failure check box indicates, that the focus stops, if there was not clear increase or decrease in the focus criterion.

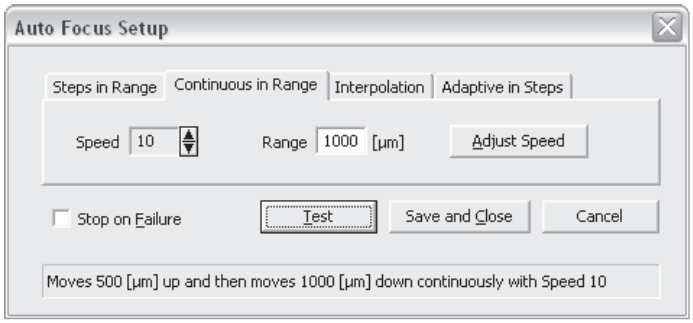
Adaptive in steps method

The **Adaptive in Steps** method moves the stage in the direction of an increasing focus criterium. The **Step** parameter defines the step in micrometers. The **Stop on failure** check box indicates, that the focusing stops if there was not a clear increase or decrease in the focus criterion. Note that objectives of different magnifications require different step size ranging from 0.3 microns (100x) to 50 microns (1x).



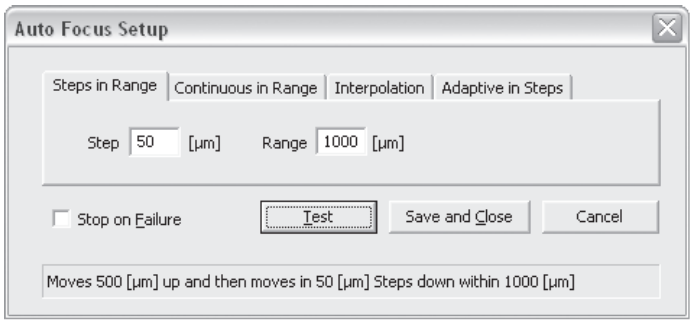
Continuous in range method

The **Continuous in Range** method (with some devices only) continuously moves the stage in a defined range. It starts moving from the bottom to the top with a constant speed. Maximal focus is searched during the movement. Depending on the camera framerate and integration, the optimal Z speed should be adjusted. Adjust the speed by the use of the **Speed** control. Press the Adjust Speed button for automatic speed adjustment. The Range parameter defines the Z travel distance in micrometers.



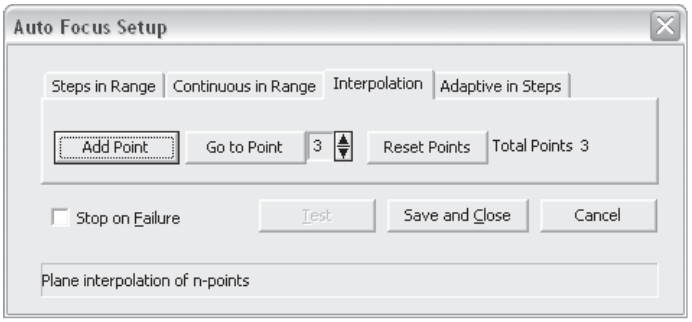
Step by step in range method

The **Steps in Range** method moves the stage within a defined range. It starts moving from the bottom to the top by a defined step. The maximal focus is searched during the movement. The Step parameter defines the Z step (the difference between two z positions). The Range parameter defines the Z travel distance in micrometers.

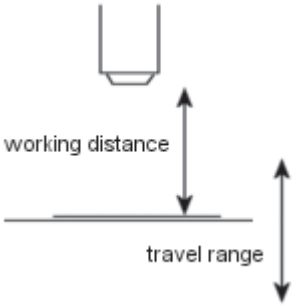


Interpolation method

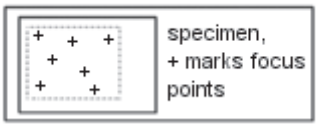
The **Interpolation** method is quite different from the other methods. It requires the XY motorization. It is able to compensate the systematical focus trends introduced by a badly adjusted microscope stage or by a sloping specimen. The algorithm must be taught before it is used. The teaching is done by moving the stage to different XY positions and by defining the focus. At least three points (minimum number to define the plane) must be added by pressing the **Add Point** button. Pressing the **Go to Point** button moves the stage to the position indicated by the number beside the button. The Reset Points button resets all defined points.



Note



When working with lower magnifying objectives (4x for example) use higher steps (like 10 microns). For higher magnifying microscopic objectives (for example 40x) use lower steps (like 3 microns). Also, the travel range should be adjusted for each objective. For example the travel range for a 20x objective should be about 100 micrometers. Make sure not to enter higher travel range than half of the working distance of the objective.



A demonstration of the interpolation focusing is shown on the picture. The area of interest on the microscopic specimen is marked by a dotted line. The focusing points are marked by „+“.

Grabbing slices

The Stage Z module enables to capture a sequence of images while the Z position is changing. When you install the Z drive to the system the **Capture Z-Series** command in becomes available from the **Acquire** menu. The functionality of this command eqals the ND - Z slices experiment definition. Please see the appropriate section of the **ND Experiments** chapter for further details.

Zoom configuration

Please, see the detailed description of the zoom configuration in the Stage XY Module chapter.

90i Module

90i microscope

The Eclipse 90i is a motorized microscope that can be operated via a PC and interacts with the camera controls. It offers a high-precision, motorized operations, focusing, observation procedures and interactive control of the microscope and digital cameras. The „Fly-eye“ lens array built into the illumination optics creates a uniform illumination throughout the visual field, perfect for digital imaging.

The Eclipse 80i is a fully manual version of 90i. The whole microscope can be divided into two parts: The 90i/80i main body and the Digital Imaging Head (DIH). There exist two versions of DIHs: fully motorized (DIH-E) and manual (DIH-M).

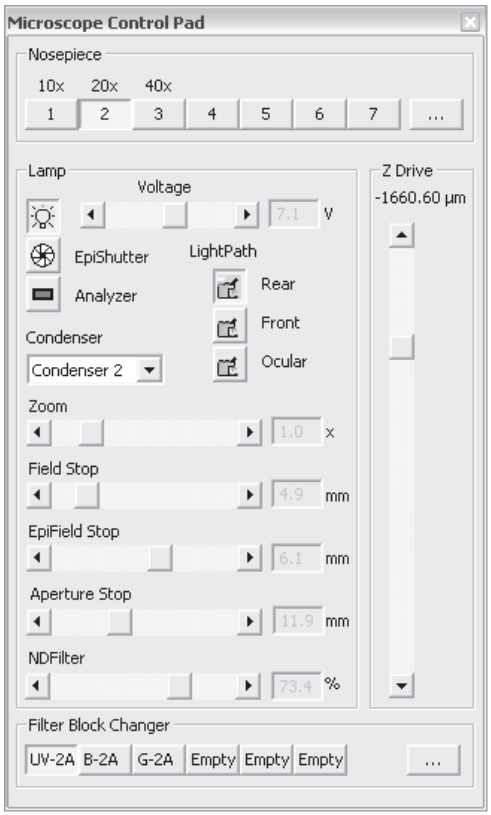
The **90i main body** contains these motorized devices: Z drive with 0.05 micron precision, Field stop, Aperture stop, Condenser, Lamp on/off and voltage driving

The **DIH-E** contains: Shutter, FilterBlock changer, LightPath changer, Zoom, EpiField stop, Analyzer.

The **DIH-M** contains a motorized shutter and other devices, that are „intelligent“. An intelligent device has no motor, but can electronically report its current position. So the DIH-M contains these intelligent devices: the FilterBlock changer, the LightPath changer and the Zoom.

Other optional devices are: XY stage - motorized, NDFilter - motorized, Nosepiece - intelligent. The DIH-E and DIH-M can be directly connected to the 90i body, but a C-Box must be used to connect to 80i. The C-Box is a „bridge“ which allows the DIH to work on the 80i microscope.

Microscope Control Pad



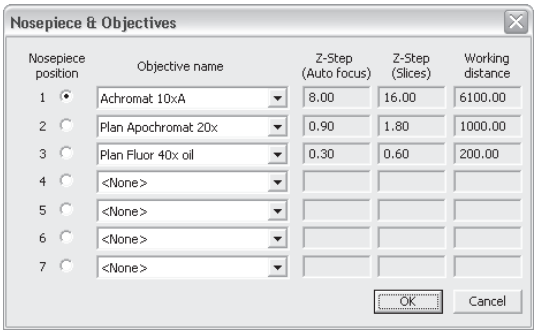
The 90i module provides control of all the components of the microscope. The microscope can be controlled through the **Microscope Control Pad,** accessible from the Devices menu. It includes the Z drive movement scroll bar; nosepiece, filter changer and light controls. Full control of the light path via the shutter, aperture and other accessories is also provided.

The Microscope Control Pad contains several sections: the Nosepiece, Lamp, Z drive and the Filter Block Changer.

Nosepiece

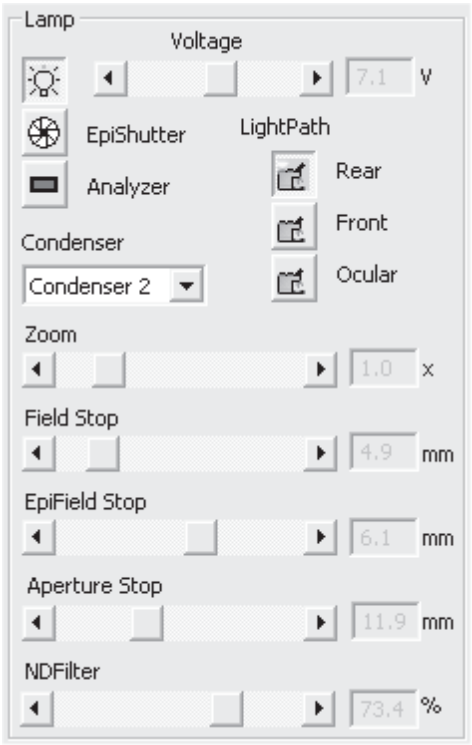
The nosepiece section of the control pad serves to control objective manipulation. There are 7 positions available - each can handle one objective. To switch objectives, just click on the button with the desiderated objective associated. To attach an objective (or to change an already attached objective) to a position, click on the „...“ button on the right side. The objectives setup window appears

Nosepiece setup



A wide range of objective types can be selected from the combo boxes to associate an actual objective with a certain position. The three parameters of the Z-steps and the Working distance on the right are not editable via this dialog window.

Lamp & Condensers



This part of the Control pad serves for the light and condenser control. The „**light**“ button switches the light on/off. The light`s intensity is adjustable via the voltage slider on the right.

The **Epi** shutter can be controlled by the associated button. The **Analyzer** button inserts/extracts the analyzer in/out of the optical light path.

The three buttons in the **Light path** section determine which light port of the microscope you are currently using. The icons represent the Rear, Front ports and the Ocular.

A **Condenser** can be changed by selecting from five possible positions in the combo box.

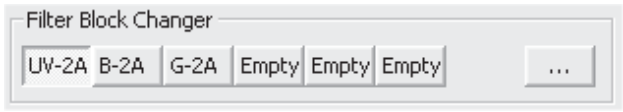
Z drive

The remaining scroll bars can serve to adjust the zoom factor, apertures diameters and the NDFilter effectiveness. If the „intelligent“ devices are used, the scroll bars cannot be moved. In this case they only display the current devices` settings.

You can move the Z drive using the slider on the right side of the Microscope Control Pad. The current position of the Z drive is indicated by the Z-axis value [µm] next to the slider.

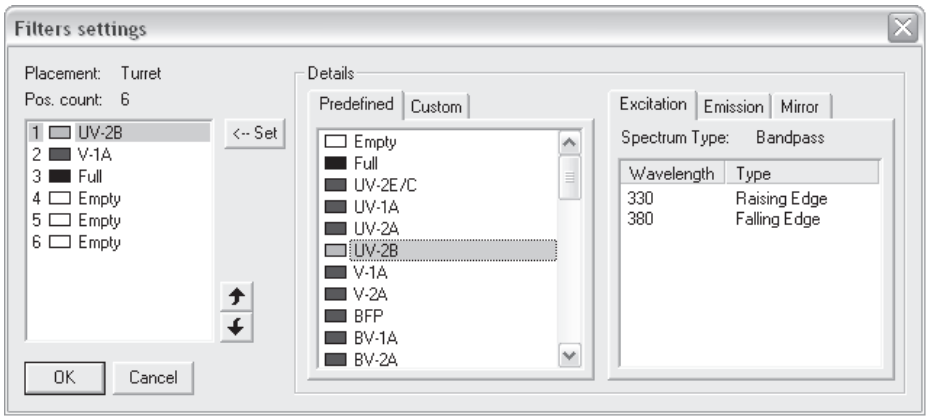
Filter block changer

You can activate filters by clicking on appropriate buttons. To change a filter position or add a new filter to the filter set, click the **„...“** button.



Changer setup

This setup window allows you to associate any filter from the Filters database or a user defined filter to a button in the Microscope Control Pad. The list of associated filters is located in the left part of the window.



When browsing filters from the Filters database, pick an actual filter that you would like to use. The details for that filter are shown in the rightmost part of the window. There you can swap between three tabs to see the type of the filter and its wavelengths characteristics.

TE2000 Module

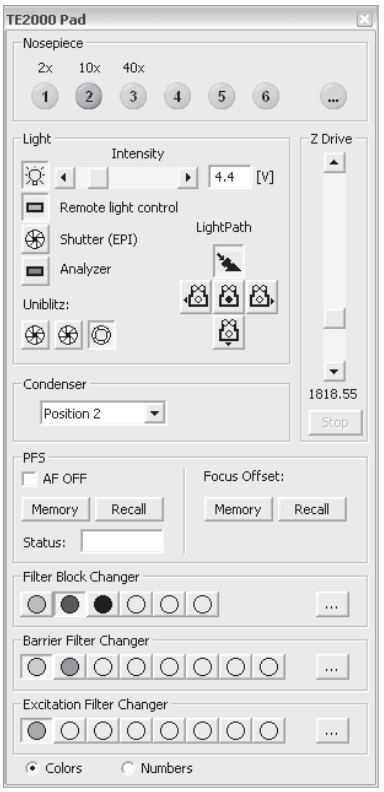
TE2000 microscope

Nikon Eclipse TE2000 is an inverted microscope to take full advantage of the infinity optical path, which allows multiple optical equipment to be used separately or simultaneously, and adds the ability to provide multiple input sources as well as output functions. TE2000 is available in three models, all featuring an exclusive multi-port design. Coupled with an extendible main body structure, all models can be flexibly configured to meet all present and future progressively advanced and diversified applications.

Microscope Control Pad

The TE2000 module provides control of all the components. The microscope can be controlled through the **Microscope Control Pad,** accessible from the Devices menu. It includes the Z drive movement controls; nosepiece, filter changers and light controls. Full control of the light path via shutters and other accessories is also provided.

The Microscope Control Pad contains several sections: the Nosepiece & Objectives, Light, Z drive, Condensers and Filter Changers`. We will examine their`s functionality consecutively.



Nosepiece & Objectives

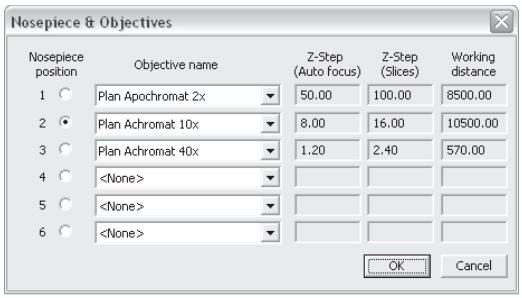
The nosepiece part of the control pad serves to control objectives manipulation. There are 6

positions available - each can handle one objective.

To switch objectives, just click on the green icon with desiderated objective. To attach an objective (or to change an already attached objective) to a position, click on the „...“ button on the right side. The objectives setup window appears

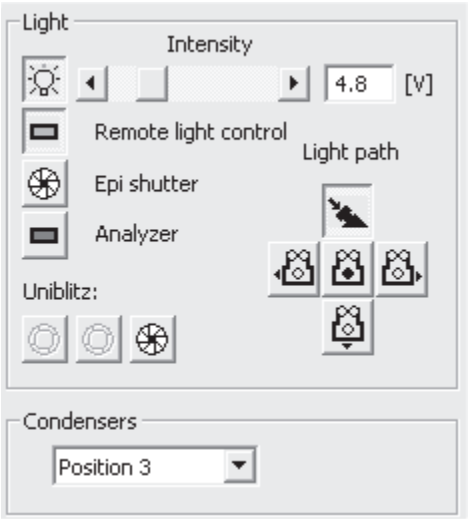
Nosepiece setup

A wide range of objective types can be selected from the combo boxes to associate an actual objective with a certain position. The three parameters of the Z-steps and the Working distance on the right are not editable via this dialog window.



Light & Condensers

This part of the Control pad serves for light and condenser controlling. The „**light**“ button switches the light on/off. The light`s intensity is adjustable via the Intensity slider on the right, or it is possible to enter the value of voltage into the editbox.



The **Remote light control** enables/disables to control the light via the Microscope Control Pad. If you prefer to control the light level manually on TE2000, you have to switch this parameter off.

All available shutters can be controlled by associated buttons. The **Analyzer** button inserts/extracts the analyzer in/from the optical light path.

All five buttons in the **Light path** section determine which light port of the microscope you are currently using. The icons represent the Ocular view and the Left, Front, Right-side and the Bottom ports.

Uniblitz shutters are optional components. The icon identifies wether the shutter is opened or closed. If the icon is disabled, no shutter is installed on that position.

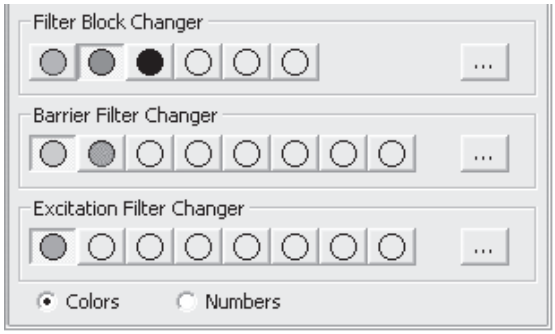
The **Condenser** can be changed by selecting from five possible positions in the combo box.

Z drive

There are two possible ways to move a Z drive. You can move it using a slider, or you can specify an exact value in µm. The Position value is indicated next to the slider.

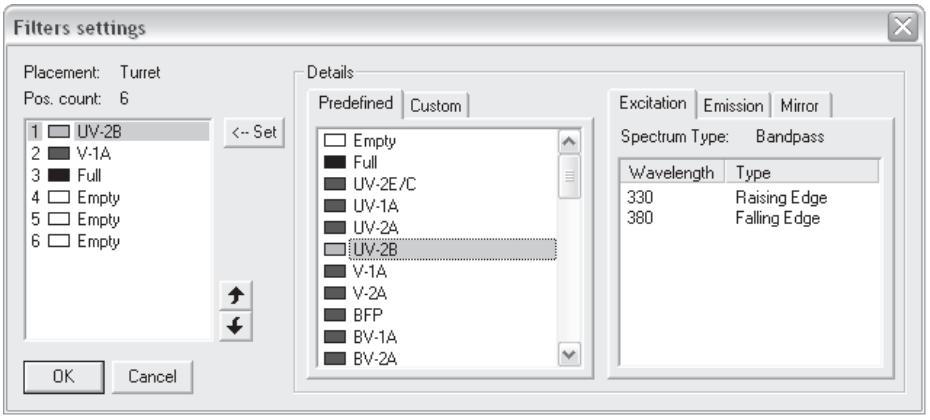
Filter changers

You can activate filters by clicking on appropriate possitions (marked by numbers, or colors corresponding to the filter colors). To change filter positions or add a new filter to your filter set, click the **Setup** button.



Changer setup

This setup window allows you to associate any filter from the Filters database or a user defined filter to a button in the Microscope Control Pad. The list of associated filters is located in the left part of the window.



When browsing filters from the Filters database, pick an actual filter that you would like to use. The details are shown in the rightmost part of the window. There you can swap between three tabs to see the type of the filter and its wavelengths characteristics.

Coolscope Module

Coolscope Overview

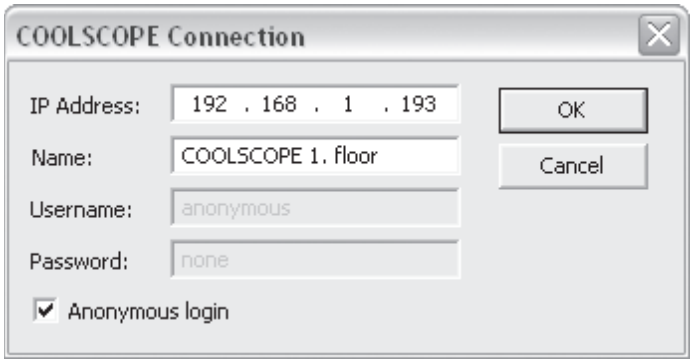
In a simple all-in-one design, COOLSCOPE includes all the functions necessary for observation, image capture and network communications features. The COOLSCOPE combines a microscope with a digital camera and network functions into one tower unit. Coolscope has a Nikon DS-5M camera integrated.

Remote access

Via a network such as Local or Wide Area Network, at different locations within the network, you can view an image being observed on the COOLSCOPE by someone else, or you can even remotely perform basic operations.

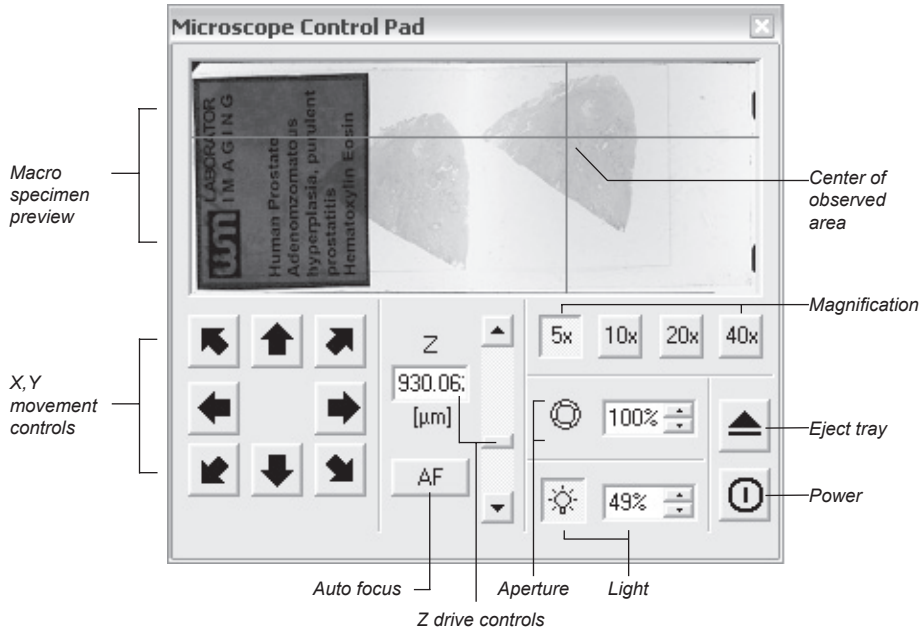
Connecting Coolscope

If Coolscope is properly connected and the Coolscope module has been installed, you will be asked to choose a grabber on startup. Pick the Coolscope grabber. After that, you will be prompted to fill in the IP address, name of the device, username, and password. Press the OK button to establish connection. Open the Device Manager via the Devices menu and click on the Add button. Add Coolscope to the list of devices and you will be ready to use it.



Coolscope control pad

Use the Microscope Control Pad command that appeared in the Devices menu after connecting the Coolscope. The control pad consists of a specimen preview frame, X,Y and Z axes movement controls, buttons representing single objectives of the nosepiece, aperture and exposure time adjust boxes. In the bottom right corner, there are the Open/close tray and Power buttons. By means of the control pad, you are able to precisely control everything but the camera.



Movement

To operate the stage, use the movement controls or click into the preview window to specify an area to observe. The red cross will move to that position.

Focusing

The focusing is done by moving the Z drive. Enter an exact value or use the scroll bar. The AF button brings the image into focus automatically.

Magnification

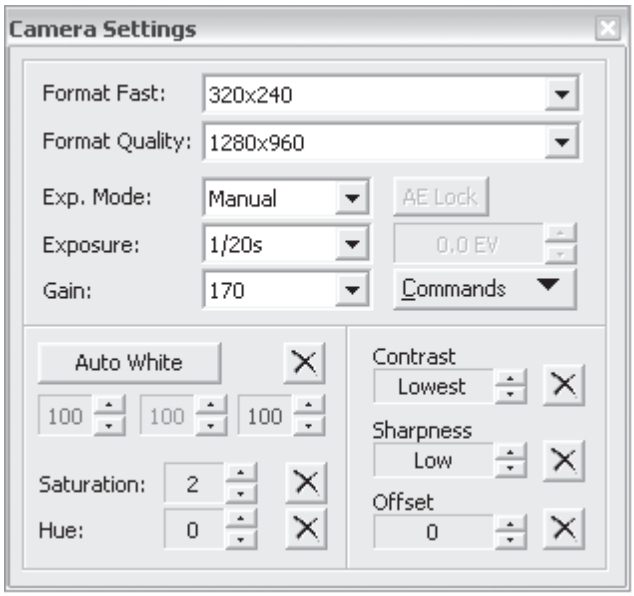
The 5x, 10x, 20x, 40x buttons represents individual objectives (magnifications). Simply click on the desired magnification button.

Illumination

The Light button switches the illumination light on/off. Its intensity is adjustable in the neighbouring editbox. The aperture size can be adjusted the same way.

Coolscope Camera

Coolscope includes the DS-5M camera as the capturing device. The following window can be invoked by the use of the Show Camera Settings command from the Camera menu.



Fast/Quality modes

You can toggle between two camera formats (Fast/Quality) and modify their properties. The first combo box offers various resolutions of the image displayed on the screen. Possible values ranges from 320x240 up to 2560x1920.

Exposure

The **Exp. Mode** combo box enables you to select from three semiautomatic and one manual exposure modes. The **AE lock** locks the exposure time currently used by one of the automatic modes. Set the exposure compensation in the nearby box.

Gain

The **Gain** value influences the image contrast. It is a multiplication constant that controls the colors dynamics (especially with lower illumination levels). Try to set a lower offset and raise the gain to enhance the contrast.

White balance

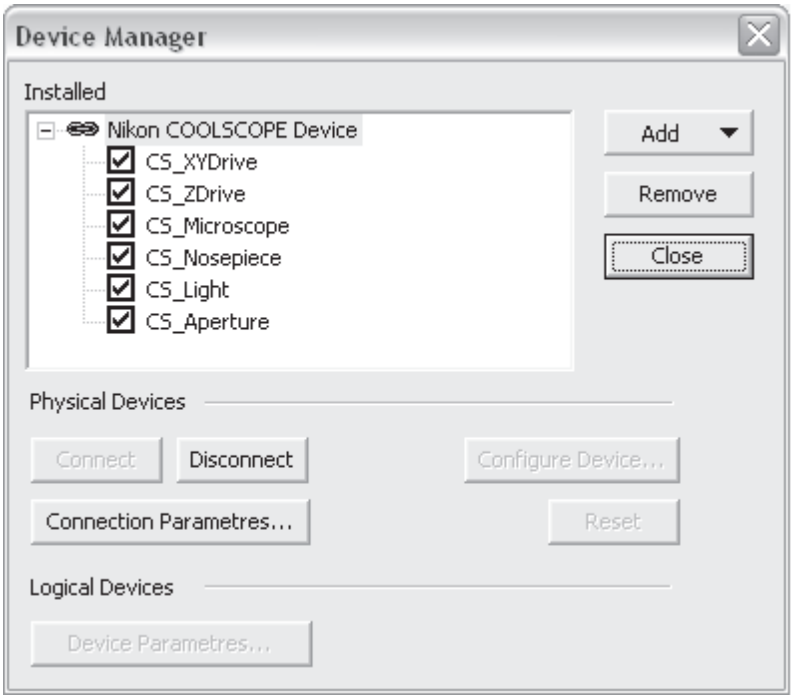
The **Auto White** button automatically adjusts individual gains of the red, green and blue components to get the color neutral white, or you can adjust the gain values manually.

Other standard digital image processes` of **Saturation**, **Hue**, **Contrast**, **Sharpness** or **Offset** adjustments can be performed.

Coolscope Devices

Coolscope is integrated direct to the core of the system as a complex device. However, it consists of number of logical devices, that can be used independently. For example, if you want to use applications, where a motorized stage is required, select the Manage Devices command from the Devices menu to open the Device Manager.

All available logical devices are listed. Check the logical devices you wish to use and control and click Close button.



Nikon MM 400/800 Microscope

Measuring microscopes

The MM-400/800 series of Measuring Microscopes incorporate key performance features expected in an advanced next generation measuring microscope:

- Greater Accuracy
- Digital Imaging and Vision Processing Metrology
- Larger Stage for Increased Workpiece Handling
- Non Contact Z-height Measurements
- Coordination with Data Processing Systems DP-E1

Logical devices

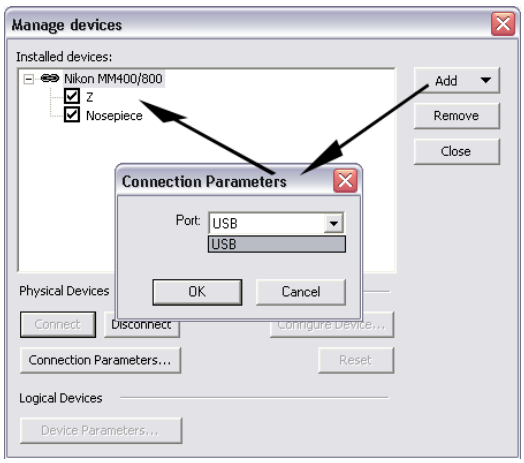
A measuring microscope connected to NIS-Elements offers two logical devices:

- **Nosepiece**
- **Z drive**

Connecting microscope

Connecting Nikon MM to NIS-Elements

- Connect the microscope to the computer via USB cable and switch it ON.
- Run NIS-Elements
- Run the **Devices > Manage Devices** command. The Device Manager dialog window appears.
- Click the **Add** button and select **Nikon MM 400/800** from the list of devices.
- Confirm the type of connection (USB). The microscope should be connected by then.
- Close the Device Manager.



Control window

Nikon MM Nosepiece & Objectives

After adding the MM microscope to the system via Device Manager, the Nosepiece logical device become available. Display the control window by the **View > Controls > Nosepiece** command. There are 5 positions available in the control window - each can handle one objective.

Nosepiece types

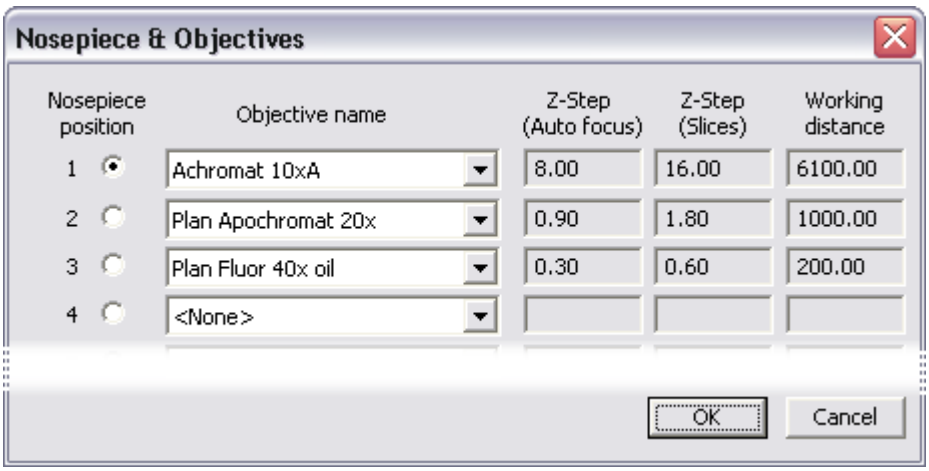
Three types of nosepiece can be attached to the microscope:

- **Manual** - cannot communicate with the computer. Each time you switch the objectives, you should also select a position in the Nosepiece control window accordingly.
- **Motorized** - cannot be controlled by the system, but when you change the objective manually, it changes the position selected in the Nosepiece control window automatically.

Objectives setup

Objectives Setup

Pressing the Setup (**...**) button on the right side, the following dialog window appears:

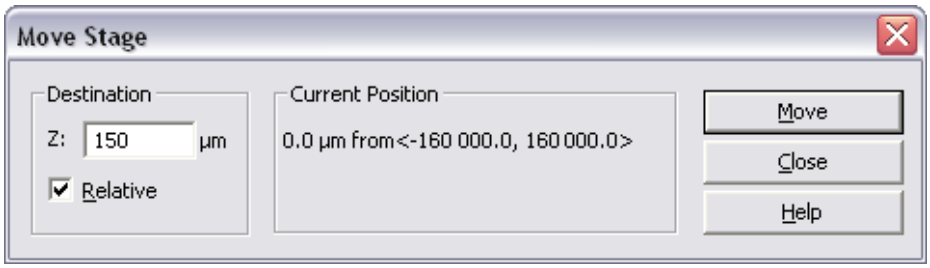


Objectives can be selected by picking from combo boxes. Each objective has its specifications displayed in the table. These specifications are not editable.

Moving Z Drive

Nikon MM Z Drive

The Z drive of the Measuring Microscopes may be moved using the **Move Z-Drive** command of the **Devices** menu. The following dialog window appears:



Moving Z Drive

The MM Z drive range is 160 mm. Users can define the zero position manually by resetting the Z axis on the microscope hardware controller.

- Select whether to define the distance **relatively** (to the current position) or absolutely (to the zero position).
- Fill in the target Z position to the edit box.
- Click the **Move** button.

The current position is indicated in the **Current Position** *section followed by the current range. Because the system is not allowed to retrieve information about the absolute position of the Z drive, the range is always set to a hypothetic* **<-160 000, 160 000>** *(double size of the real range) every time the origin is reset to zero.*

Nikon Eclipse LV Series Microscope

LV Series

The ECLIPSE LV Series realizes an unprecedented level of versatility based on the new concept module design.Digital imaging function such as capture, analysis, and database formation - are growing faster than ever before.The LV motorized systems addresses to these demands and come with mechanisms that automatically optimizes observation technique and illumination, and these settings can be controlled from external devices.

Logical devices

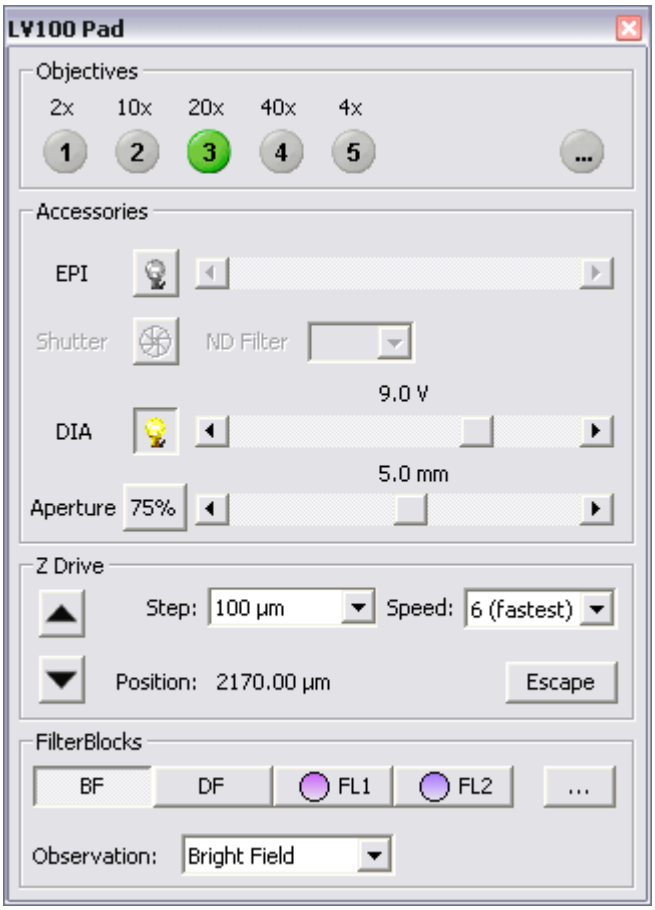
The LV microscope control pad offers the following controls:

- **Accessories**
- **Filters**
- **Nosepiece**
- **Z drive**

Microscope control pad

LV - Control Pad

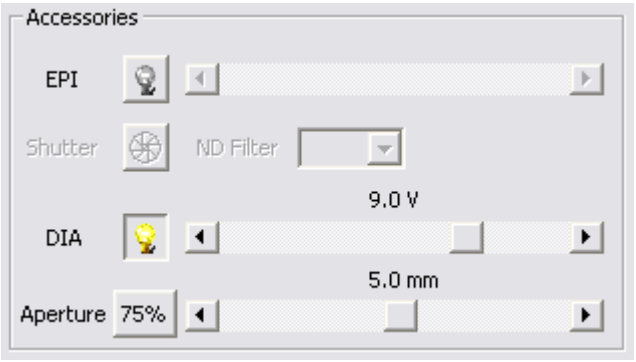
A Control Pad offers all necessary functions of microscope control. The objective changer, lights, aperture, Z drive, filter changer and You can display it by selecting the **Microscope control pad** command from the Devices menu, or by it's keyboard shortcut (**Ctrl + Alt + M**). Each section of the pad is described separately on the following pages.



Light control

Control Pad - Accessories

This part of the control pad serves for controlling light sources and related accessories.



Light path settings

EPI

Click the nearby icon and the EPIscopic illumination will be activated. Voltage of the lamp can be adjusted using the slider on the right.

Shutter, ND Filter

These buttons become enabled when the **X-Cite 120 Fluorescence Illumination** is attached to the system. They handle the inbuilt shutter and ND filter. The ND filter can be set to 0, 12, 25, 50, and 100% of transmitted light.

DIA

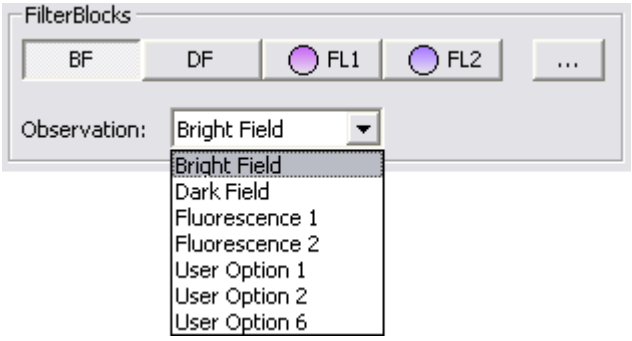
Click the nearby icon and the DIAscopic illumination will be activated. Voltage of the lamp can be adjusted using the slider on the right.

Aperture

The slider adjusts size of the aperture. The **75%** button adjusts the the aperture to 75% of the current objective pupil. Therefore the actual aperture size after the button is clicked is objective dependent.

Filter changer

Control Pad - Filters

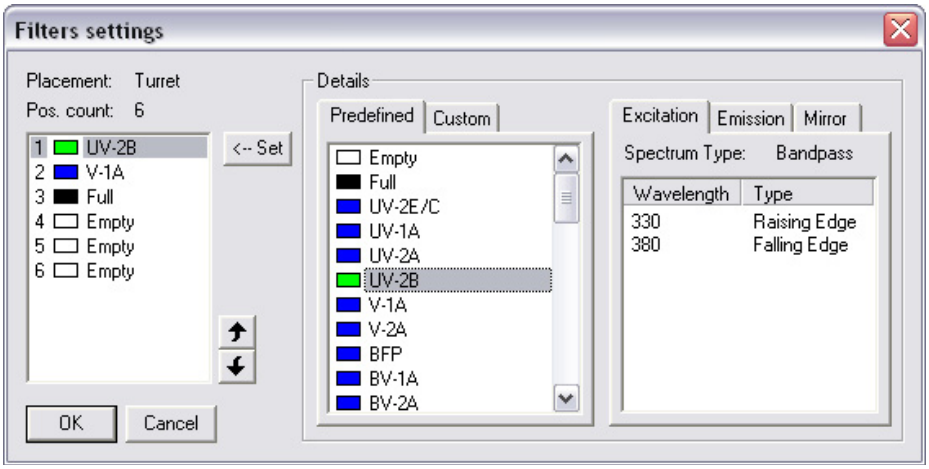


This part of Control pad serves for filters controlling. There are 4 available positions in the changer. The first two have fixed filters for bright field (BF) and dark field (DF) microscopy. The other two are free for any filters.

Filters setup

Filters Setup

This area is dedicated to filter mangement. You can activate filters by clicking on appropriate positions. To change filter assignment or add a new filter to the filter set, click the Setup (**...**) button. The following dialog box appears:



When browsing filters from the **Filter database** their details are shown in te most right part of this window. You can swap between three panes to see type of the filter and wavelengths characteristics.

To assign a filter to the filter position:

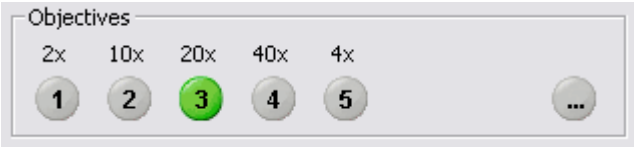
- Select one of the available positions which the filter will be assigned to.
- Select the filter name from the database of filters.
- Click the **<-Set** button

The filters can be moved within the available positions using the **Up/Down** buttons.

Objective changer

Nosepiece & Objectives

This part of Control pad serves for controlling objectives manipulation. There are 5 positions available - each can handle one objective.

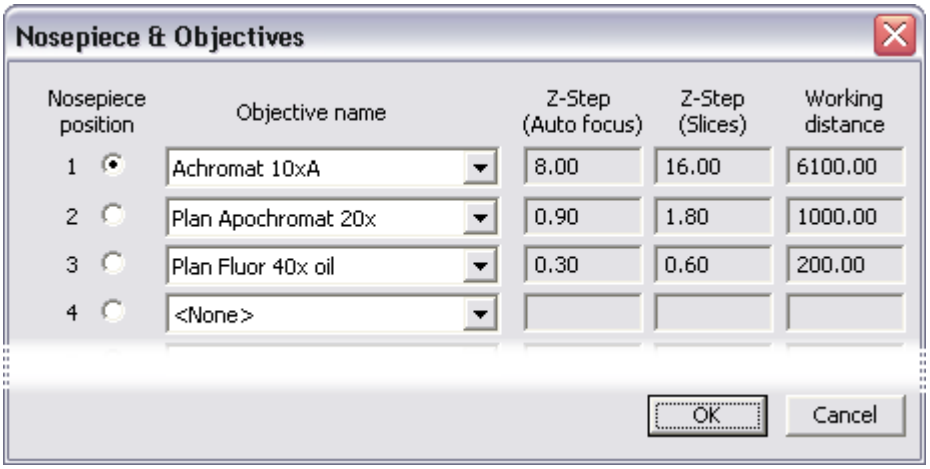


To switch objectives, just click on the button with desiderated objective.

Objectives setup

Objectives Setup

Pressing the Setup (**...**) button on the right side, the following dialog window appears:

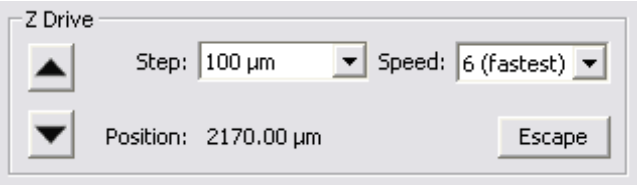


Objectives can be selected by picking from combo boxes. Each objective has its specifications displayed in the table. These specifications are not editable.

Z drive pad

Control Pad - Z Drive

This section controls the Z Drive.



Controls

Up/Down Arrows

The arrow buttons aside move the Z drive up or down.

- Click the buttons **once**, the Z drive moves up/down by the defined **Step**
- Keep the buttons pressed, the Z drive moves continuously by the defined **Step**
- Hold the **Shift** key down at the same time, and the **Step** value is doubled automatically.
- Hold the **Ctrl** key down at the same time, and the **Step** value is halved automatically.

Step

Select the desired step value from the combo box. There are the following step values available: **Continuous**, **0.1**, **1**, **10**, **100**, **1000 µm**.

Speed

There are 7 gears of the Z motor predefined. Select one of them.

Escape/Refocus

After you press the **Escape** button, the objective will move up in order for the specimen to be operated easily. Return the objective to its original position by pressing the same button whose name changed to **Refocus**.

Nikon AZ100 Microscope

AZ100 introduction

Nikon AZ100 Multizoom microscope is an ideal choice for bioscience and industrial applications where multi-purpose wide field of view to high magnification applications are common. The AZ100 microscope features unique to the industry telecentric optics which creates images of the same size for objects at any distance and has a constant angle of view across the entire field.

Key features

Key features of AZ100 include:

- a wide field of view
- long working distances
- a high resolution capability
- a broad range of observation techniques are supported (EPI/DIA-DIC, DIA-BF & Oblique, EPI-FL, EPI-RL EPI/DIA-Simple POL)
- a wide magnification range from 5 to 500x

Logical devices

Logical Devices

The AZ100 microscope connected to NIS-Elements offers the following logical devices:

- **Nosepiece**
- **Filter changer**
- **Z drive**
- **Zoom**

When the microscope is connected to the system, the **AZ100 Microscope Control Pad** become available (in the [Devices] menu).

Connecting microscope

Connecting Nikon AZ100 to NIS-Elements

- Connect the microscope to the computer via USB cable and switch it ON.
- Run NIS-Elements
- Run the [Devices > Manage Devices] command. The Device Manager dialog window appears.
- Click the [Add] button and select [Nikon AZ100 microscope] from the list of devices.
- Close the Device Manager.

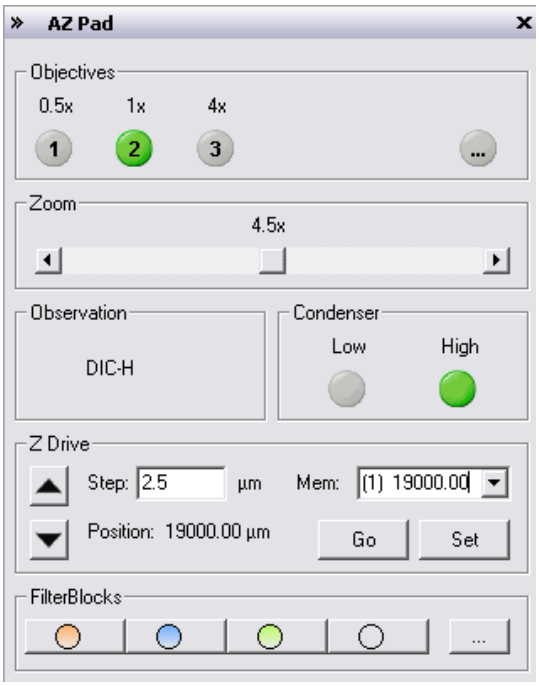
When AZ100 is connected to NIS-Elements, the manual remote control is disabled automatically.

Microscope control pad

AZ100 Microscope Control Pad

The AZ100 Microscope Control Pad consists of the following portions:

- **Objectives**
- **Zoom**
- **Observation**
- **Condenser**
- **Z Drive**
- **Filter Blocks**



Objectives

The objectives portion of the pad indicates the nosepiece status: the currently selected objective and objectives magnification. The objective changer cannot be controlled by NIS-Elements.

Zoom

Zoom magnification can be changed by moving the scroll bar. The current zoom factor is indicated above.

Condenser

The green button indicates the current condenser lense position (Low/High). The condenser cannot be controlled.

Z Drive

The Z drive can be controlled via the microscope control pad in the following way:- The [up/down arrows] move the Z drive by a defined [Step].

- The current position can be memorized by pressing the [Set] button. The position value will be written to the pull-down menu.
- To move the Z drive to a memorized position, select the desired value from the pull-down menu and press the [Go] button.
- The current position is indicated below the [Step] edit box.

FilterBlocks

The currently selected filter of the four available filter positions is indicated in the Filter Blocks portion of the pad. The filter changer cannot be controlled by NIS-Elements. Filters settings may be changed after pressing the [...] button.

En Symbol for separate collection applicable in European countries

This symbol indicates that this product is to be collected separately.
The following apply only to users in European countries.
- This product is designated for separate collection at an appropriate collection point. Do not dispose of as household waste.
- For more information, contact the retailer or the local authorities in charge of waste management.

De Symbol für getrennte Wertstoff-/Schadstoffsammlung in europäischen Ländern

Dieses Symbol zeigt an, dass dieses Produkt separat entsorgt werden muss.
Folgendes gilt für Verbraucher in europäischen Ländern:
- Dieses Produkt darf nur separat an einer geeigneten Sammelstelle entsorgt werden. Eine Entsorgung im Hausmüll ist unzulässig.
- Wenden Sie sich für nähere Informationen bitte an Ihren Händler oder die örtlich für Abfallentsorgung zuständigen Behörden.

Fr Symbole pour la collecte sélective applicable aux pays européens

Ce symbole indique que ce produit doit être collecté séparément.
Les mesures suivantes concernent uniquement les utilisateurs européens.
- Ce produit doit être jeté séparément dans un point de collecte approprié. Ne jetez pas ce produit dans une poubelle réservée aux ordures ménagères.
- Pour plus d'information, contactez le détaillant ou les autorités locales responsables de la gestion des ordures.

Es Símbolo para recogida separada aplicable en países Europeos

Este símbolo indica que este producto se recogerá por separado.
Lo siguiente sólo se aplicará en países Europeos.
- Este producto ha sido designado para su recogida en un punto de almacenamiento apropiado. No lo tire como un deshecho doméstico.
- Para más información, contacte con el vendedor o autoridades locales al cargo de la gestión de residuos.

Dk Symbol for special bortskaffelse af denne type produkter i de europæiske lande

Dette symbol angiver, at dette produkt skal bortskaffes specielt.
Det efterfølgende er kun til forbrugere i de europæiske lande.
- Dette produkt skal bortskaffes på fx en genbrugsplads el .lign. Det må ikke smides væk som normalt husholdningsaffald.
- For yderligere information kontakt din forhandler eller de lokale myndigheder, som fx teknisk forvaltning.

Nl Symbool voor gescheiden inzameling zoals dat wordt gebruikt in Europese landen

Dit symbool betekent dat dit product apart moet worden ingezameld.
Het volgende is alleen van toepassing op gebruikers in Europa
- Dit product dient gescheiden ingezameld te worden op een daartoe bestemd inzamelpunt. Niet wegwerpen bij het normale huisvuil.
- Neem voor meer informatie contact op met het verkooppunt, of met de lokale instantie die verantwoordelijk is voor het verwerken van afval.

Pt Símbolo para recolha de resíduos em separado utilizado nos países Europeus

Este símbolo indica que este produto é para ser recolhido separadamente.
Esta norma aplica-se só para os utilizadores nos países Europeus.
- Este produto está designado para recolha de resíduos em separado num recipiente apropriado. Não deitar no caixote do lixo doméstico.
- Para mais informações, contactar o revendedor ou as autoridades locais responsáveis pela gestão dos resíduos.

It Simbolo per la raccolta differenziata applicabile nei paesi europei

Questo simbolo indica che il prodotto va smaltito separatamente.
La normativa che segue si applica soltanto agli utenti dei paesi europei.
- Il prodotto è designato per lo smaltimento separato negli appositi punti di raccolta. Non gettare insieme ai rifiuti domestici.
- Per maggiori informazioni, consultare il rivenditore o gli enti locali incaricati della gestione dei rifiuti.

This symbol is provided for use in the People's Republic of China, for environmental protection in the fields of electronic information products.

このマークは、中国のお客様に向けたもので、電子情報製品分野における環境保護を目的としています。

No Symbol for kildesortering i europeiske land

Dette symbolet indikerer at produktet skal kildesorteres.
De nedenstående punktene gjelder for alle europeiske brukere.
- Dette produktet skal kildesorteres og innleveres til dedikerte innsamlingspunkter. Må ikke kastes med normalt husholdningsavfall.
- For mer informasjon, ta kontakt med din forhandler eller lokale myndigheter.

Se Symbol för separat upphämtning i euopeiska länder

Den här symbolen anger att produkten måste hämtas separat.
Följande gäller bara användare i europeiska länder.
- Den här produkten är avsedd för separat upphämtning vid ett lämpligt uppsamlingsställe. Produkten får inte kastas i hushållsavfall.
- För mer information, kontakta återförsäljaren eller de lokala myndigheter som ansvarar för avfallshantering.

Fi Erillisen keräyksen merkki Euroopan maissa

Tämä merkki osoittaa, että tuote kerätään erikseen.
Seuraavat maininnat koskevat vain eurooppalaisia käyttäjiä.
- Tämä tuote kerätään erikseen asianmukaisista keräyspisteistä. Älä hävitä tuotetta talousjätteiden mukana.
- Lisätietoja saat jälleenmyyjältä tai paikallisilta jätehuoltoviranomaisilta.

Ru Символ сортировки мусора, использующийся в европейских странах

Данный символ означает, что этот продукт должен утилизироваться отдельно от других.
Приведенная ниже информация касается только пользователей из стран Европы.
- Данный продукт должен утилизироваться отдельно от других в соответствующих приемных пунктах. Не выбрасывайте данный продукт вместе с бытовым мусором.
- Дополнительную информацию Вы можете получить у продавца или у местных властей, отвечающих за утилизацию мусора.

Gr Σύμβολο για την ξεχωριστή αποκομιδή απορριμμάτων στις Ευρωπαϊκές χώρες

Αυτό το σύμβολο υποδηλώνει ότι η αποκομιδή αυτού του προϊόντος πρέπει να γίνει ξεχωριστά.
Τα κάτωθι απευθύνονται μόνο σε Ευρωπαίους χρήστες.
- Αυτό το προϊόν είναι σχεδιασμένο έτσι ώστε να γίνεται η αποκομιδή του σε ειδικά σημεία. Μην το πετάτε μαζί με τα υπόλοιπα απορρίμματα.
- Για περισσότερες πληροφορίες, επικοινωνήστε με τον διανομέα του προϊόντος ή με τις υπεύθυνες τοπικές αρχές για θέματα διαχείρισης απορριμμάτων.

Pl Symbol oznaczający segregowanie odpadów, stosowany w krajach Europy

Ten symbol oznacza, że produkt musi być wyrzucany oddzielnie.
Poniższe uwagi mają zastosowanie tylko dla użytkowników z Europy.
- Ten produkt jest przeznaczony do oddzielnej utylizacji i powinien być dostarczony do odpowiedniego punktu zbierającego odpady. Nie należy go wyrzucać z odpadami gospodarstwa domowego.
- Aby uzyskać więcej informacji, należy skontaktować się z przedstawicielem przedsiębiorstwa lub lokalnymi władzami odpowiedzialnymi za zarządzanie odpadami.

Hu Európai országokban érvénes "Elkülönített hulladékgyűjtés" jelzése

Ez a jelzés azt jelenti, hogy ezt a terméket elkülönítve kell gyűjteni.
Az alábbiak csak az európai országokban élő felhasználókra érvényes.
- Ezt a terméket a megfelelő hulladékgyűjtőhelyen, elkülönítve kell gyűjteni. Ne dobja ki háztartási hulladékként.
- További információkért forduljon a forgalmazóhoz, vagy a helyi hatóság hulladékgyűjtésért felelős részlegéhez.

Cz Symbol pro oddělený sběr odpadu platný v evropských zemích

Tento symbol znamená, že tento produkt se má odkládat odděleně.
Následující pokyny platí pro uživatele z evropských zemí.
- Tento produkt se má odkládat na místě sběru k tomuto účelu určeném. Neodhazujte spolu s domácím odpadem.
- Více informací o způsobu zacházení s nebezpečným odpadem vám podá příslušná místní instituce.

Jp ヨーロッパにおける廃棄物個別回収のシンボルマーク

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インストルメンツカンパニー 産業機器統括部 営業部

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